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Sulphur-Containing Amino Acids of Wheat Proteins

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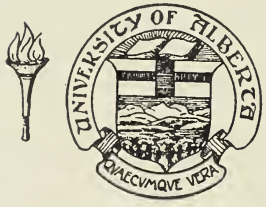

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The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance a thesis on "The sulphur-containing amino acids of wheat proteins," submitted by R. Kasting, B. Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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Examiner

Examiner

Examiner

THE SULPHUR-CONTAINING AMINO ACIDS OF WHEAT PROTEINS

ROBERT KASTING
Department of Plant Science

A THESIS

submitted to the University of Alberta
in partial fulfilment of the requirements
for the degree of

MASTER OF SCIENCE

Edmonton, Alberta
April, 1948

THE DOCTRINE-CONCEPT CURVE AND
ITS USES IN CHILD GUIDANCE

ROBERT SPRING
Superintendent of Child Guidance

A THESIS

submitted to the University of Florida
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Gainesville, Florida
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THE SULPHUR-CONTAINING AMINO ACIDS OF WHEAT PROTEINS

Robert Kasting

INTRODUCTION

Wheat flours with similar protein content often produce bread loaves of different sizes because of differences in their protein quality. Rigby (34) has attributed differences in quality in a single wheat variety to the total sulphur or sulphur-containing amino acids present in the protein. He showed that, whereas the quantity of crude protein would not account for loaf volume differences, the total sulphur content of wheat, flour and gluten was significantly correlated with loaf size. Further, he showed that the cystine content of the gluten is highly correlated with the sulphur content of the wheat and therefore the cystine in the gluten was believed to contribute to baking strength.

In a further study of the sulphur and wheat quality relationship, Gerbrandt (19), using five varieties of wheat grown on different soil types, showed that the percentage of sulphur in the whole wheat was a better measure of baking

THE RUBBER-CONTAINING FRACTION FROM
CERTAIN PLANTS

Robert Spurling

INTRODUCTION

strength than was percentage crude protein. In addition his work showed that cystine and especially methionine content of the crude gluten accounted for more of the variability in loaf volume than did sulphur in wheat.

In these two studies, analysis for sulphur-containing amino acids was carried out only on the crude gluten washed from flour. It was thought that accurate methods for the determination of cystine and methionine in whole wheat and flour would provide a new means of attack, which would be of value in studies of the role of sulphur in flour quality. A direct method for cystine and an indirect method for methionine were found to be applicable to the determination of these amino acids in flour and wheat.

Wheat was grown on the Breton gray soils and was fertilized similarly to that used by Rigby (34). This material was available for further studies on the relationship between sulphur and loaf volume. Because of an early frost, the 1946 wheat graded poorly and produced very poor bread which did not show the expected variations in loaf size. It was therefore thought that investigations relating primarily to baking strength would be of little value. Nevertheless, as this material had a wide range in sulphur content it was admirably suited for investigations of nitrogen, sulphur, cystine and methionine relationships. A study of these relationships is the primary concern of this thesis.

The data for loaf size of the samples investigated were available. The loaf volumes for the 1947 material varied considerably. This provided an opportunity to compare loaf volume relationships with the results of other work from this laboratory.

As Rigby and Gerbrandt have shown that the sulphur-containing amino acids appear to affect wheat flour quality, a study of factors affecting the cystine and methionine content of wheat and flour might provide further information on the whole problem.

There is very little information in the literature as to the effects of various fertilizer treatments on the ultimate amino acid content of cereal grains. This knowledge would be of importance in protein nutrition, where quality as well as quantity of proteins is recognized as being important. Some of the material used in this study provides such information with respect to the sulphur-containing amino acids in wheat.

LITERATURE REVIEW

Harrow (25) lists four sulphur-containing amino acids, namely methionine, cystine, lanthionine and djenkolic acid. He mentions the compounds cysteine and ergothioneine; the former is classified as a reduced form of cystine of importance in

biological reactions, the latter as a derivative of histidine.

Glutathione, a polypeptide of glutamic acid, glycine and cysteine, is of importance in a consideration of sulphur-containing amino acids because of the sulphur present in the sulphydryl group.

In the literature reviewed, no reference reported the presence of lanthionine, djenkolic acid or ergothioneine in wheat or wheat products. The sulphur-containing compounds which have been reported in wheat are the amino acids cystine and methionine, the vitamins thiamine and biotin and the compounds cysteine and glutathione. The sulphur in the latter two is usually determined and reported as cystine-sulphur.

Greaves and Bracken (23) found that the thirty-six wheats investigated by them did not contain sulphur in the sulphate form. Woodman and Evans (40), however, report samples of bran and middlings with sulphate sulphur contents of 0.004% and 0.01% respectively.

Numerous studies have been made on the thiamine content of wheat (5). The paper of Johannson and Rich (27) gives a general indication of the thiamine content of wheat. These authors found that the amount of thiamine in wheat from the three prairie provinces in 1940 ranged from 2.2 to 8.0 μg per gram with a mean of 3.93 μg.

Bailey (5) reports the biotin content of two samples of wheat as 22 millimicrograms and 113 millimicrograms per gram.

It is obvious that this organic sulphur-containing compound is present in wheat in very small amounts.

There are numerous papers which report values for the sulphur-containing amino acids in wheat and wheat products but most of the values reported are on isolated samples of wheat, flour or gluten.

A systematic study of the cystine content of different varieties of wheat and a single variety grown under various cultural conditions is reported by Gubler and Greaves (21). Using a colorimetric method for the cystine determination they found that the cystine-sulphur constituted about one-half the total sulphur of wheat. They observed that in general, variations in sulphur content are accompanied by corresponding variations in cystine content. In addition nitrogen content was highly correlated with percentage cystine. The correlation coefficients reported for sulphur and cystine and nitrogen and cystine are 0.83 and 0.75 respectively.

Gubler and Greaves also observed that, in general, as the total sulphur and cystine content increased there was a decrease in the percentage of total sulphur represented by cystine. This was also observed by Wood (38) in a study of leaf proteins. These observations may be attributed to incomplete estimation of cystine at the higher levels of cystine content.

Csonka (13) determined the cystine contents of various salt extractions of Marquis, Tenmarq and Fulhio wheats. He found

the total cystine contents to be 0.27%, 0.17% and 0.16% respectively. Although he does not report the percentage sulphur, the nitrogen values are 3.31%, 2.58% and 2.34% in the order of the varieties. Csonka concluded that the amino acid content is different in the different varieties of wheat studied.

Rigby (34) in a study of the effect of sulphur on the quality of wheat grown on sulphur deficient soils found that cystine is the principal form of sulphur in gluten. In the samples examined he found the percentage of cystine varied from 56% to 89% of the total sulphur. The mean value was 76%. The protein content of this material was not an adequate indicator of the baking strength. The sulphur content of the wheat, however, was positively and significantly correlated with loaf volume. The gluten contained most of the sulphur in the wheat and larger amounts of sulphur were present in glutens from strong flours. The cystine content of the gluten was related to loaf volume, sulphur in gluten and sulphur in wheat. From Rigby's data there does not appear to be a decrease in the amount of sulphur represented as cystine when the cystine content increases.

Rigby found that the sulphur content of the flour follows closely that of the whole wheat but the level is slightly higher. This was taken as evidence that sulphur is more concentrated in the endosperm of the kernel than in the whole wheat.

Gerbrandt (19), in a study of the distribution of sulphur in wheat, reported that although samples of Marquis wheat were significantly higher in sulphur than were samples of Thatcher, there was very little difference in the gluten cystine values. The methionine contents of the glutens were, however, constantly and significantly lower in Thatcher than in Marquis.

In this study the simple correlation coefficients reported for cystine or methionine in gluten with loaf volume, protein in wheat and sulphur in wheat are highly significant.

Using direct methods for both the cystine and methionine determinations Gerbrandt found the sulphur of these amino acids accounted for 101.4% of the total sulphur of gluten from Marquis wheat grown at six stations. The distribution of sulphur in gluten was also determined for Marquis and Thatcher grown at two stations. The total cystine and methionine sulphur represented a mean recovery of 97% of the total gluten sulphur. The gluten sulphur represented a mean of 70.5% of that found in the flour and 62.2% of that in the whole wheat.

In the second of his papers on amino acids in staple foods, Csonka (14) states that although the protein content of patent flour is lower than that of the whole wheat flour, there is a larger percentage of cystine in the flour. A direct determination on a sample of whole wheat flour gave a value of 0.17% cystine. The values for the flour, bran and shorts of this wheat are respectively 0.17%, 0.01% and 0.02%.

D. Breese-Jones (10) reports little difference in the cystine content of whole wheat and a patent flour milled from the wheat. This worker reports a methionine content of 0.48% for a sample of flour milled from wheat containing 0.10% methionine. Obviously the results as reported are incorrect or the method used for the methionine determination is not consistent. The total methionine according to these results has actually increased in the flour. This is hardly possible.

Harrison, Thomas and Hill (24) traced the movement of sulphur in the wheat kernel by the use of radioactive sulphur. Radiographs of the grain showed the greatest concentration of sulphur was in the embryo. There appeared to be a fairly uniform concentration of sulphur in the starchy endosperm; but at the periphery of the endosperm there was a much higher concentration. This is especially so in the aleurone layer and the cells immediately underneath. The aleurone cells contain a large part of the protein of the bran. They are mostly removed as part of the bran in the milling process.

Barton-Wright (6) made a study of the distribution of amino acids in the wheat grain. Using a microbiological technique he found that, apart from the germ, the highest concentrations of 11 amino acids including cystine and methionine occur in the outer part of the grain.

Block and Bolling (8) report cystine contents for five whole wheats. The values range from 0.16% to 0.29%. Three

flour samples are reported with cystine contents of 0.31%, 0.26% and 0.27%. Block and Bolling also report on cystine values obtained by other workers for germ, bran and shorts.

Padva (32) in a study of the amino acid content of gluten reports a value of 1.43% cystine.

Workers reporting methionine results on isolated samples of wheat are Reisen, Schweigert and Elvehjem (35) and Chitre and Keni (12). The first group of workers used a microbiological method, the second group used a colorimetric technique.

Grau and Almquist (22) compared a colorimetric and a biological method for the determination of methionine in wheat, wheat protein and wheat bran. They found a close correlation between the two methods.

Wood (38) states that the only sulphur-containing components of the protein molecule are the amino acids cystine and methionine. Further, Wood says these acids may occur in a free state in plant tissues with cysteine and glutathione. The latter two compounds are derived from free cystine and methionine or from the protein molecule.

Cysteine and glutathione are important because of their active sulphydryl groups.

Sullivan, Howe and Schmalz (37) have shown that glutathione is concentrated in the wheat germ and that patent flour contains only traces if any of this compound.

Myers and Working (31) state that sulphydryl groups are present in proteins in a free or reactive form and/or a bound

or non-reactive form. The reactive groups cause the protein when in the native form to give a positive nitroprusside test. The non-reactive form will only give a positive reaction with the nitroprusside reagent if the protein is first denatured to liberate the sulphydryl group. Meyers and Working tested gluten for the foregoing groups and concluded that neither free nor bound sulphydryl groups were present. The water-soluble proteins from wheat flour were found to contain non-reactive sulphydryl groups which are made reactive on denaturation.

An interesting paper by Blumenthal and Clarke (9) reports that in some proteins sulphur may exist in forms other than cystine, cysteine and methionine. These authors suggest that at least two other sulphur-bearing constituents are present, one of which could be the compound ergothioneine.

Baernstein (2) working with thirty-two different proteins estimated total sulphur and the sulphydryl, disulphide and methionine fractions. He reports that the three fractions accounted for roughly 101.5% of the total sulphur but values varied from 85.9% to 117.8%.

Bailey (4) states that of the known sulphur-containing amino acids, only cystine and methionine have been isolated from protein hydrolysates. Bailey compared specific methods for cystine and cysteine with methods for cystine which included any additional -S-S-groups which may be present in the protein molecule. He found a disparity between the two estimations. Although the author suggests that there may be losses of cystine

due to decarboxylation or deamination of a portion of the cystine during hydrolysis, or inhibition of color used in the colorimetric determination of substances present in the protein hydrolysate, he does not preclude the possibility of the presence of sulphur-containing amino acids other than cystine and methionine.

In a later paper Baernstein (3), using newer methods, determined the cystine and methionine in eleven proteins. He obtained an average recovery of 96.9% of the total sulphur as the aforementioned amino acids and H_2S. The latter compound was attributed to the presence of inorganic sulphate.

Blumenthal and Clarke (9) found that cystine can be completely oxidized to sulphate by boiling with fuming nitric acid, but synthetic methionine yields only small amounts of sulphate. Using solutions of cystine or methionine separately and together with gelatin, these authors report a very high recovery of cystine-sulphur as sulphate.

Mazur (30) applied this method for cystine to a highly purified Limulus hemocyanin. He found that the nitric-acid-oxidizable sulphur gave values which compared with those obtained using two classical colorimetric methods.

Lugg (29) in 1938 used the oxidizing method for the determination of cystine in a study of the sulphur distribution of protein preparations from leaves of different grass species. Lugg, assuming that the only sulphur-containing amino acids in

protein are cystine and/or cysteine and methionine, used the following relationship:

cystine-S. + cysteine-S. + methionine-S. + other S. = total S.

As "other S." was believed to be present entirely as sulphate it should appear as inorganic SO_4 on acid hydrolysis.

Lugg compared a specific colorimetric procedure and Baernstein's HI digestion method for cystine with his so-called "differential-oxidation procedure." He concluded that the latter method, although less specific than the other quantitative methods, appears to yield more appropriate results. He warns, however, that to accept these results is to accept the assumption that the sum of the fractions thus estimated necessarily equals the total sulphur.

Using a principle similar to that of Lugg, Callan and Toennies (11) found that alkaline-permanganate solution oxidized cystine-sulphur but not methionine-sulphur to sulphate. These authors applied this method to the estimation of cystine in egg albumin.

Block and Bolling (8) reviewed the methods for cystine determinations up to 1945. They suggest that the "differential-oxidation procedure" appears to have special value when estimations of cystine are to be made on protein preparations heavily contaminated with carbohydrate.

Evans (17) in 1945 made a further study of the "differential-oxidation procedure." He oxidized mixtures of pure cystine

and/or methionine, with dextrose, soybean oil meal and fish meal. Almost all of the cystine-sulphur but very little of the methionine-sulphur was recovered as sulphate. Evans' data suggest that considerable organic matter must be present to completely oxidize cystine to sulphate.

The values determined for methionine by the "differential oxidation procedure" and a colorimetric method on samples of soybean oil meal and fish meal compare favorably. A comparison of the "differential-oxidation procedure" with other methods showed the cystine and methionine values reported for the former method are respectively higher and lower. The differences could be accounted for by losses of cystine upon acid hydrolysis where this step is taken or a difference in the composition of the materials studied.

Evans (18) and coworkers in a later study applied the "differential-oxidation procedure" to the determination of cystine and methionine in dry peas. This paper is of interest because of the application of this method and because a study was made of the influence of fertilizers and cultural practices on the cystine, methionine and protein contents of dry peas.

Evans et al. report that the addition of sulphur-containing fertilizers to the soil increased the percentage of total sulphur but decreased the protein content of the peas. The protein of peas fertilized with ammonium sulphate or gypsum contained significantly more sulphur than did the protein of

peas fertilized with sodium nitrate. The authors conclude that cystine values are influenced in the same direction as the sulphur but the methionine values were not significantly different.

Evans and coworkers found very small differences in the effect of maturity, climatic conditions and variety on the cystine and methionine contents of peas.

Greaves and Bracken (23) studied the relationship of sulphur to nitrogen in a group of spring and winter wheats. They found that the nitrogen and sulphur contents of twenty-one wheat varieties were closely associated. A correlation coefficient of 0.81 was reported.

Greaves and Bracken also studied the sulphur-content of Kanred wheat under varying cultural conditions. The data presented show that ploughing under of legumes will result in slight increases in the nitrogen content of wheat grown on such land. The sulphur content did not vary consistently.

Gubler and Greaves (21), investigating the same material as Greaves and Bracken, report that variations in cultural treatments cause at least small variations in cystine content.

Smith and Wang (36) made a study of the effects of environmental factors on the cystine and methionine contents of the vegetative parts of four species of grasses. They conclude that the effects of manuring are irregular and are relatively unimportant. Lime did not alter the cystine or methionine to a significant extent. Ammonium sulphate added to

rye-grass late in the growing stage effected small reductions in the cystine and methionine content.

Wood and Barrien (39) state in a study of the sulphur metabolism of plants, that the sulphate ion constitutes the sole source of sulphur to plants and the sulphur-containing proteins are the synthetic end-products in the sulphur metabolism. These workers obtained the following results with Sudan grass. The application of increased amounts of potassium sulphate in solution to plants did not affect the quantity of cystine-sulphur isolated. Inorganic sulphate - sulphur in the leaves was increased with increasing amounts of sulphate fertilizer. The ratio of protein-nitrogen to protein-sulphur remained approximately the same. Increases in ammonia-nitrogen increased the contents of cystine and protein sulphur as well as amino-nitrogen and protein-nitrogen, but decreased the sulphate-sulphur content. These workers suggest that ammonia acts as a limiting factor in the formation of protein sulphur from sulphate.

Barrien and Wood (7) working with leaves at different stages during the life cycle of the plant show that the ratio of protein-nitrogen to protein-sulphur is higher, the greater the nitrogen treatment. If the initial amount of sulphate supplied to the plants remained unchanged, increased nitrogen treatments were found to increase protein-sulphur.

Peters (33) worked with a number of crop plants including wheat grown on the fertilized plots of the Breton gray soils and Edmonton black soils. He found that at the Breton station, where

the natural soil sulphur is low, the percentage sulphur of grains and legumes from plots treated with sulphur-containing fertilizers was greater than that of crops from the non-sulphur fertilized plots. Wheat from the Edmonton station did not show an increase in sulphur on the application of a sulphur-containing fertilizer. This was taken as evidence that sulphur is not limiting at Edmonton.

MATERIALS

The material used in these experiments was from three sources:

Series I - Marquis flour, 1944.

Flour was milled from individual samples of Marquis wheat grown in replicated rod-row plots at Athabaska, Bon Accord, Fallis, Warburg, Sundre and at Edmonton on fallow and stubble. A composite sample of Marquis from the Rust Research Laboratory, Winnipeg, was also used. These samples were from the same lots of flour as was the material used by Gerbrandt (19).

Series II - Thatcher wheat and flour, 1946 and 1947.

Thatcher wheat was grown on the Breton Experimental Field in the gray-wooded soil area of Alberta. Through the courtesy of the Department of Soils, University of Alberta,

sufficient wheat was made available for milling, baking and other analysis. A complete detailed history of the Breton experiment can be obtained from papers by Wyatt, Newton and Ignatieff (41), Peters (33), and Kenwood (28).

The wheat for the analysis reported in this paper was harvested from the following fields.

Series E, 1946 - Part of this field, plots 1 to 11, has been continuously cropped with wheat since 1929, except for two years when the field was fallowed and one year when barley was grown. The rest of the field, plots 13 to 26, has been cropped continuously with wheat since 1939.

Series B, 1946 - The wheat was grown after clovers which had occupied the land for two years in a five year rotation.

Series C, 1947 - This field was the wheat after clover crop for 1947.

Each of the foregoing series consists of twenty-six plots which are variously fertilized. The total amounts of fertilizers applied to all plots for each series are listed in Table I, page 19, of Kenwood's paper (28). During the summer of 1947, no fertilizers were added to the plots.

Wheat from twenty-one of the twenty-six plots in each of the above three series was used for the determinations reported in this thesis. In addition the wheat was milled into flour and further determinations were carried out. All of the

samples from plots one to eleven were used, the checks 1, 5 and 11 being bulked to give a representative check sample. Ten samples from plots 13 to 26 were selected because of the relative amounts of nitrogen and sulphur fertilizers used. The samples were 13, and 18 to 26 inclusive. The check plots 13 and 18 were bulked to give a single check sample representative of a part of the field. Samples 22 and 26 were bulked for the same reason.

Table I shows the Breton plot numbers, the sample numbers used throughout this paper, the fertilizers used on each plot and the grades of the harvested samples.

Table II gives the approximate composition of the fertilizers used in experiments on the gray-wooded soils.

TABLE I

Key to sample numbers and the grades of Thatcher wheat grown at Breton

Plot No.	Sample No.	Fertilizer	Grades 1946 Series E	1946 Series B	1947 Series C
1,5,11	1	Check	2,3,2	5,5,4	5,5,5
2	2	Manure	2	4	6
3	3	16-20 + K_2SO_4	2	5	6
4	4	Ammonium Sulphate	2	3	6
5	5	Lime	2	4	5
7	6	Lime + Triple Phos.	2	4	5
8	7	Triple Phos.	3	4	5
9	8	Manure + 16-20	3	4	5
10	9	16-20	3	4	5
13,18	10	Check	3,2	4,4	5,5
19	11	Ammonium Nitrate	2	3	5
20	12	Ammonium Nitrate + Gypsum	2	4	5
21	13	Gypsum	3	4	6
22,26	14	Check	3,3	3,3	5,*
23	15	Sodium sulphate	3	3	5
24	16	Potassium sulphate	3	3	5
25	17	Ammonium sulphate + potassium sulphate	3	3	5

*Lost

TABLE II

Approximate composition of fertilizers used in the gray-wooded soils experiments

Fertilizer	N %	P_2O_5 %	K %	S %	Gypsum Equivalent %
Triple superphosphate	1	43	-	1	6
Ammonium phosphate (16-20)	16	20	-	14	-
Ammonium sulphate	20	-	-	24	-
Gypsum	-	-	-	18	95
Sodium sulphate (dry)	-	-	-	22	-
" " (hydrated)	-	-	-	11	-
Potassium sulphate	-	-	40	16	-
Lime	-	-	-	-	-
Ammonium nitrate	34	-	-	-	-

Table 1

Log Fe sample locations and log values
of Timbalier Island Core GC 71B20

Sample	Log Fe	Description
1		Water
2		Water
3		1ft-2ft + Mud
4		Mud
5		Mud
6		Mud + shell fragments
7		Shells + Mud
8		Shells
9		Shells + 1ft-2ft
10		1ft-2ft
11		Mud
12		Mud
13		Mud
14		Mud
15		Mud
16		Mud

Series III - Water Culture Solutions

The general procedure used for growing plants in culture solutions is given by Hoagland (26). The method for growing wheat was modified somewhat. Following is a brief description of the procedure, equipment and solutions used in the experiments with wheat.

Four tanks were used in each experiment. The larger tanks, each with a capacity of 225 liters of solution and with openings for 125 corks, were used in experiments I and II. In experiments III and IV, each tank held 64 corks and had a capacity of 110 liters of solution. Each cork contained two or three holes in which the wheat plants were placed.

The wheat used throughout all the experiments was the variety Red Bobs. The seed was germinated and grown on a germinating screen. When the plants reached a height of 1 1/2 to 3 inches they were placed in the corks so that the roots were immersed in nutrient solution.

The nutrient solutions used in the experiments were based on Hoagland's solution (26) but modifications were made to suit each experiment. All the chemicals excepting the iron tartrate, calcium di-hydrogen phosphate and the A-Z solution were made up as one-molar stock solutions. The concentrations of the stock solutions of iron tartrate and calcium di-hydrogen phosphate were 0.5% and 0.05 M respectively. The A-Z solution was made up as suggested by Hoagland.

The volumes of stock solutions used for each tank are given in Tables III and IV.

Experiment I - Table III gives the composition of the complete and sulphur-deficient solutions. The wheat plants of all four tanks were placed in complete nutrient solutions for the first two weeks after transplanting. At two week intervals the complete solutions of tanks 1, 2 and 3 were replaced by the sulphur-deficient solution. Plants in tank 4 were grown throughout on a complete solution.

TABLE III

Volumes of stock solutions added to each tank containing approximately 225 liters of water

Chemical	Concentration	Complete Solution Volume c.c.'s	Solution Lacking Sulphur Volume c.c.'s
KH_2PO_4	1 M	225	225
KNO_3	1 M	1125	1325
$CaNO_3$	1 M	1125	900
$MgSO_4$	1 M	450	--
$Mg(NO_3)_2$	1 M	--	450
A-Z solution	--	225	225
Fe Tartrate	0.5%	*	*

*1 c.c. of stock solution per litre of water about once or twice a week.

As the plants in this experiment were transplanted in September, they did not mature until the latter part of December. From approximately October 22 to the time of harvest, it was necessary to supplement daylight with artificial illumination.

Experiment II - Experiment I was carried out in the greenhouse. Considerable trouble was experienced from mildew, fungus-gnats, and other pathogenic conditions. Because of the greenhouse hazards it was decided to repeat the experiment with the tanks out-of-doors. The plants were grown during the summer months from May 5 to September 15.

Experiment III - Solutions were made up in four tanks to supply the following four conditions:

Tank 1 - low sulphur, low nitrogen.

Tank 2 - high sulphur, low nitrogen.

Tank 3 - low sulphur, high nitrogen.

Tank 4 - high sulphur, high nitrogen.

The volumes of stock solutions for each tank are given in Table IV.

TABLE IV

Volumes of stock solutions added to tanks containing 110 litres of distilled water

	High Nitrogen High Sulphur Tank 4	High Nitrogen Low Sulphur Tank 3	Low Nitrogen High Sulphur Tank 2	Low Nitrogen Low Sulphur Tank 1
	Volume cc's	Volume cc's	Volume cc's	Volume cc's
KH_2PO_4	110	110	660	660
KNO_3	560	560	-	-
$Ca(NO_3)_2$	560	475	12	-
$MgSO_4$	225	6	210	6
$Mg(NO_3)_2$	-	170	-	12
A-Z	110	110	110	110
$Ca(H_2PO_4)_2$	-	-	10,800	4,200
Fe Tartrate	110*	110*	110*	110*

*Iron tartrate is added two or three times a week according to the foliage color.

The material was grown in the greenhouse from May 5 to July 29, 1947.

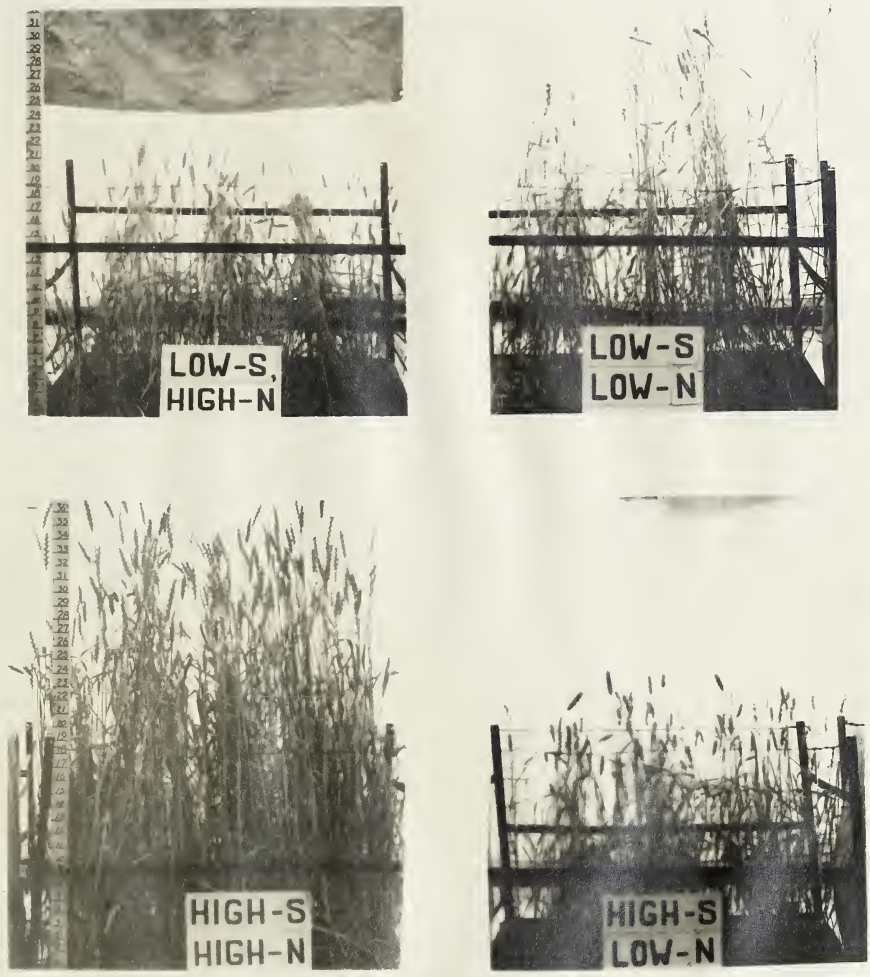
Yield data were not taken because of extensive damage by sparrows. Plate I shows the relative heights and density of growth of the wheat at harvest.

TABLE IV

Volumes of stock solutions used to titrate
containing 110 l Lres of dimethyl water

	Tank 1	Tank 2	Tank 3
	High Pressure High Sulphur	Low Sulphur High Hydrogen	High Sulphur Low Hydrogen
	Volume cc's	Volume cc's	Volume cc's
$K_2Cr_2O_7$	110	110	660
$KMnO_4$	560	560	-
$Ca(NO_3)_2$	560	475	15
$NaNO_3$	285	0	210
$Mg(NO_3)_2$	-	170	-
A-1	110	110	110
$Ca(C_2H_3O_2)_2$	-	-	$10,000
Fe Tartrate	110#	110#	110#

PLATE I



Relative heights and density of growth
for experiment III.

Experiment IV - Experiment III was repeated in the greenhouse from August 5 to December 3, 1947. Tanks 3 and 4 were discarded because of very poor growth.

Two months after transplanting, when about one-quarter of the wheat was in head, sulphur was completely eliminated from Tank 2.

Artificial light was used to supplement daylight from the last week in October until harvest.

The plants became badly infested with mildew and were therefore treated with sulphur fumes on September 24. Twice during the growing season nicotine sulphate was sprayed in the greenhouse to destroy aphids.

METHODS

Statistical Analysis

The statistical methods described by Goulden (20) are used throughout this study.

Gluten Washing

Four percent mono-phosphate adjusted to pH 6.8 by the addition of sodium di-phosphate, as suggested by Dill and Alsburg (15), was used as a stock solution. The actual washing solution consisted of the stock solution diluted with unboiled distilled water to a 0.1% concentration. Thirty-gram samples

of flour were washed for 12 minutes with a stream of solution delivered at approximately 100 ml. per minute.

As much water as possible was squeezed from the samples by hand, before wet weights of gluten were recorded. Weights of the dry gluten were obtained after drying in vacuo at 98°C. for approximately 24 hours.

Baking

Flour samples of 100 grams were baked using the malt-phosphate-bromate formula, with 0.3% diastatic malt, 0.1% ammonium phosphate, and 0.001% potassium bromate. Single bakes were carried out on each Breton sample because of the small quantity of flour.

Nitrogen

Nitrogen was determined by the Kjeldahl-Gunning-Arnold method, with mercuric oxide as a catalyst. The results are reported as percentage of the dry matter.

Sulphur

The A.O.A.C. method (1), using nickel crucibles, open fusion with sodium-carbonate and sodium-peroxide as modified by Rigby (34) was used for sulphur determinations.

Cystine and Methionine

It was desirable to find a method for cystine and methionine that could be applied to materials containing large amounts of carbohydrate and other non-protein materials. The differential-

oxidation procedure, as used by Evans (17) on soybean meal, was found to be satisfactory for wheat and wheat products.

Preparation of the sample

The whole wheat was ground to a fine meal in a Wiley mill. The dried gluten samples were ground in a burr mill.

The samples of wheat, flour and gluten were oven dried for 1 hour at 130°C. Duplicate samples of about 4 grams of wheat and flour or 0.5 grams of gluten were accurately weighed.

Determination and Calculation of Cystine

The samples were boiled with concentrated nitric acid in a hydrolyzing flask under a reflux condenser for 12 to 16 hours. Fifteen millilitres of concentrated nitric acid (specific gravity 1.61) were added for every one gram of material. After oxidation the clear, yellow hydrolyzate was transferred to a 600 ml. beaker, and one-half a gram of KNO_3 was added. The solution was evaporated to dryness on a steam-bath. To eliminate any nitrates, which would interfere with the subsequent sulphate determination, two 10 cc. portions of concentrated HCl were added and evaporated.

The dry residue was dissolved in approximately 300 ml. of distilled water and 1 ml. of concentrated HCl. The solution was boiled and filtered into a 600 ml. wide-mouthed erlenmeyer flask. The remaining procedure is identical with the determination of sulphates in any solution.

The calculation of cystine percent is as follows:

$$\text{Cystine } \% = \frac{\text{Weight of } BaSO_4 \times 51.47}{\text{Weight of sample}}$$

Calculation of Methionine

Assuming that cystine-sulphur and methionine-sulphur make up the total sulphur of the wheat, flour or gluten, we can calculate the methionine content as:

Methionine-sulphur = Total sulphur minus cystine-sulphur.

The foregoing procedure for the determination of cystine and methionine in wheat, flour or gluten is based on two assumptions:

(1) Cystine and methionine are the only sulphur compounds present in significant quantities in wheat and wheat products. (Cysteine and glutathione sulphur are considered as part of the cystine-sulphur fraction.)

(2) Cystine-sulphur is oxidized to the sulphate form. The methionine-sulphur is not oxidized to sulphate-sulphur.

With reference to the first assumption, a review of the literature showed that, besides the sulphur-containing amino acids, thiamine and biotin are the only organic sulphur compounds whose presence has been reported in wheat. These substances are present in such small amounts that the sulphur contributed by them would not affect the significance of the results reported in this thesis.

Allowances must be made for sulphate-sulphur if it is present in the wheat. It is generally known that if sulphate-sulphur is present in the wheat kernel it is in very small amounts. In all the literature reviewed there were no reports of sulphate-sulphur in wheat. Woodman and Evans (40), however, reported

small amounts of this sulphur form in bran and middlings. The method of Woodman and Evans for sulphate-sulphur was applied to some samples of the wheat and flour used in this study. As no sulphate-sulphur was detected in the samples surveyed, no further attempts were made to determine this sulphur-fraction.

Gerbrandt (19) used methods completely different from those reported in this paper. He accounted for all of the sulphur as cystine and methionine in a number of samples of crude gluten.

The assumption that cystine-sulphur is oxidized to sulphate-sulphur and methionine is not, was validated by Evans (17) and Blumenthal and Clarke (9). Evans carried out recovery experiments of pure cystine and methionine from soybean and fish meals. Blumenthal and Clarke did similar experiments with gelatin.

To test the "differential-oxidation procedure" further, determinations were made on samples of flour and samples of flour to which cystine or methionine had been added.

In the recovery experiments reported in Table V, different amounts of cystine were added to a commercial flour with a known cystine content. The flour and cystine mixture was oxidized for approximately 15 hours with 7 ml. of nitric acid per gram of flour. As shown by results presented later better oxidation and purer precipitates of barium sulphate can be obtained with larger amounts of nitric acid. The regular cystine determination was carried out and the results are reported in Table V.

TABLE V

Cystine recovery from a commercial flour.

Flour	Cystine added	Theoretical cystine	Actual cystine recovered	Recovery % of Theoretical
gms.	mgms.	mgms.	mgms.	
1.000	0	--	4.25)4.15 4.05)	
1.000	18.75	22.90	(a) 20.59 (b) 21.51 (c) 21.57 (d) 21.93	(a) 89.9 (b) 93.9 (c) 94.2 (d) 95.8
2.500	12.50	22.88	(a) 22.18 (b) 22.34 (c) 23.62 (d) 21.72	(a) 96.9 (b) 97.6 (c)103.2 (d) 94.9
4.000	5.00	21.60	(a) 21.46 (b) 22.48	(a) 99.4 (b)102.7
*5.000	12.50	33.25	(a) 33.25 (b) 35.10	(a)100.0 (b)105.6
6.000	20.00	64.90	(a) 66.60 (b) 67.22	(a)102.6 (b)103.6
10.000	25.00	66.40	(a) 66.22 (b) 69.10	(a) 99.7 (b)104.1

*Flour acid ratio 1:8

The results presented in Table V show that cystine-sulphur is quantitatively oxidized to sulphate-sulphur. Except for the one-gram sample, the actual recoveries of cystine added to flour are within 6% of complete recovery. The results for the one-gram sample are low. Evans showed that in recovery experiments of this type considerable organic matter must be present to get a complete oxidation of cystine-sulphur to sulphate-sulphur. The one gram sample confirms this observation.

TABLE V

Cystine recovery from a commercial flour.

Flour Added gms.	Cystine Added mgms.	Theoretical Cystine Present mgms.	Actual Cystine Recovered mgms.	% of Theoretical Recovery
1.000	0	--	1.25 (a) 1.06 (b)	1.45
1.000	16.75	21.20	20.59 (a) 21.4 (b) 21.47 (c) 21.43 (d)	97.8 99.5 99.4 95.0
2.500	34.70	41.25	22.16 (a) 22.34 (b) 23.64 (c) 21.72 (d)	96.4 97.2 103.2 94.5
4.000	54.90	61.60	24.16 (a) 24.48 (b)	90.4 92.7
*5.000	12.50	13.25	12.45 (a) 13.40 (b)	100.0 105.4
6.000	20.00	44.90	46.60 (a) 47.22 (b)	102.4 103.4
10.000	29.50	46.40	46.22 (a) 47.10 (b)	99.7 102.1

*Flour ash ratio 1:8

The results presented in Table V show that crystine sulphur is quantitatively oxidized to sulphate-sulphur. Except for the one-gram sample, the actual recoveries of crystine added to flour are within 5% of complete recovery. The results for the one-gram samples are low. Other reported tests in recovery experiments of this type considerable organic matter must be present to get a complete oxidation of crystine-sulphur to sulphate-sulphur. The one gram sample confirms this observation.

To test whether methionine-sulphur is oxidized to sulphates, different amounts of methionine and flour were treated with nitric acid. The procedure for this experiment was the same as for the experiment with cystine. The results are reported in Table VI.

TABLE VI

Methionine recovery from a commercial flour.

Flour	Methionine* added	Theoretical cystine	Actual cystine recovered	Recovery % of Theoretical
gms.	mgms.	mgms.		
1.000	0	--	4.25) 4.15 4.05)	
1.000	10.00 (16.10)	4.15	1.44 5.30	34.7 127.7
2.500	6.25 (10.06)	10.38	10.86 10.04	104.6 96.7
4.000	2.50 (4.03)	16.60	16.08 15.18 15.24	96.9 91.4 91.8
5.000	2.50 (4.03)	20.75	20.85 22.24	100.5 107.2
**1.000	0	--	4.35) 4.34 4.32)	
5.000	3.75 (6.03)	21.70	21.26 20.43	98.0 94.1

*Cystine equivalent in brackets.
**A new standard flour.

The results in Table VI show that methionine-sulphur is not oxidized to sulphate-sulphur under these conditions.

Flour	Sulphonic added	Theoretical cystine	Actual cystine recovered	% of Theoretical recovery
1.000	0	--	1.25 1.05	1.15
1.000	10.00 (10.00)	4.15	1.44 5.20	34.7 125.3
2.500	6.25 (10.00)	10.38	10.66 10.04	102.6 96.7
4.000	2.50 (4.00)	16.60	16.08 15.10 15.28	96.9 91.3 92.8
5.000	2.50 (4.00)	20.75	20.25 22.80	128.5 107.5
1.000	0	--	1.25 1.35	1.30
5.000	3.75 (6.00)	23.70	23.26 20.48	98.2 86.4

Cystine equivalent in proteins.
Wet pea starched flour.

The results in Table VI show that sodium-sulfur is not oxidized to sulphonic-sulphur under these conditions.

To test whether sodium-sulphur is oxidized to sulphates, different amounts of methionine and flour were treated with nitric acid. The procedure for this experiment was the same as for the experiments with cystine. The results are reported in Table VI.

TABLE VI

Methionine recovery from a commercial flour.

In most of the experiments the cystine recovered is within 6% of the actual amount present. Where the actual recovery is not within 6% of the theoretical, the values are generally lower. If the methionine-sulphur was oxidized the recovery would be much more than indicated in the table.

The results reported in Table VI suggest that the cystine of the flour is not completely oxidized. The nitric acid : flour ratio is very much smaller than that used by other workers and might account for the incomplete oxidation of cystine.

Because the oxidation of cystine apparently was not completed in some of the foregoing cases, experiments were carried out to find the best conditions for complete oxidation. The results of different oxidation times and different nitric acid : flour ratios on the oxidation of flour cystine were studied.

Table VII shows the effect of different amounts of nitric acid on the oxidation of flour cystine. Five-gram flour samples were oxidized for approximately 15 hours with different volumes of nitric acid.

TABLE VII

The effect of different volumes of nitric acid on the oxidation of flour cystine

Ratio of gms. flour to mls. acid	Mgms. of cystine per gram flour	Remarks
1-4	3.61) 3.79)	Barium Sulphate precipitate black. Hydrolyzate dark brown.
1-5	4.11	Barium Sulphate precipitate yellow-brown. Hydrolyzate brown.
1-6	4.52	Dirty-white precipitate. Hydrolyzate yellow.
1-7	4.51	Dirty-white precipitate. Yellow hydrolyzate.
1-8	4.77	White precipitate. Hydrolyzate light yellow to white.
1-9	4.57	"
1-10	4.85	"
1-11	4.88	"
1-12	4.58	"

At ratios higher than 1:7 results are reported as averages of duplicate determinations that check within 5%. With flour:acid ratios below 1:8 there was considerable variation between duplicates and as a result more than two determinations had to be made at these ratios.

Generally, the results show that increases in nitric acid volume to 8 ml. per gram of flour bring about increases in the sulphate-sulphur recovered. At the lower acid concentrations

TABLE VII

The effect of different volumes of nitric acid on the solubility of silver cyanide

Ratio of conc. nitric acid to conc. HCl	Norm. of cyanide per liter	Remarks
1-4	2.02 1.75	Barium chloride precipitate. Clear. Hydrolysis very slight.
1-5	1.11	Barium chloride precipitate yellow-brown. Hydrolysis great.
1-6	.92	Silky-white precipitate. Hydrolyzes yellow.
1-7	.81	Silky-white precipitate. Yellow supernatant.
1-8	.77	White precipitate. Hydrolyzes light yellow to white.
1-9	.67	"
1-10	.65	"
1-11	.56	"
1-12	.50	"

the barium sulphate precipitate varies in color from black to a dirty white. This indicates that at these concentrations the oxidation is not complete and there may be considerable occlusion of impurities. Above the 1:8 ratio the precipitate is white.

It was desirable to observe the effect of still higher concentrations of acid on the amount of sulphate-sulphur recovered.

Oxidations were carried out on a new standard flour with flour : acid ratios ranging from 1:7 to 1:17. The results are reported in Table VIII as averages of duplicates which check within 5%. The oxidation time was approximately 15 hours.

TABLE VIII

Effect of flour : acid ratios on the sulphate-sulphur determined

Ratio gms. flour to mls. acid	Mgms. cystine per gram flour
1:7	4.10
1:9	4.25
1:11	4.35
1:13	4.28
1:15	4.07
1:17	4.19
Mean	4.21 ± 0.11

Table VIII suggests that there are small differences in sulphate-sulphur determined above the flour : acid ratios of 1:7. At the ratios reported in the table it is assumed that

Ratio wea. flour to wea. soyabean	Mean oyabean flour
1:7	4.10
1:9	4.25
1:11	4.35
1:13	4.28
1:15	4.07
1:17	4.19
Mean	4.21 ± 0.11

cystine is oxidized completely to sulphate with a hydrolysis time of 15 hours.

In view of the foregoing, determinations of cystine for this paper were carried out with flour : acid ratios of 1:15. Exceptions to this ratio are noted.

The effect on the quantity of sulphate-sulphur measured, as a result of different oxidation times, is presented in Tables IX and X.

Tables IX and X give the results of different hydrolysis times at flour : acid ratios of 1:6 and 1:7 respectively. The results are presented as averages of duplicates which check within 5%. The flours in each table are from a different lot.

TABLE IX

Comparison of various hydrolysis times on the sulphate-sulphur determined (Flour : acid ratio 1:6)

Time	Mgms. cystine per gram flour	Remarks
2	4.56	Precipitate brown to black.
4	4.62	Precipitate brown to black.
6	4.58	Precipitate brown to black
8	4.37	Precipitate brown.
15	4.52	Dirty white precipitate.

TABLE X

Comparison of various hydrolysis times on sulphate-sulphur determined. (Flour : acid ratio 1:7)

Time	Mgms. cystine	Color of precipitate
2	4.38	
4	4.28	In all cases
6	4.30	brown to black
8	4.27	

The data presented in the tables suggest that time of oxidation over 2 hours has little effect on the sulphate-sulphur determined. The precipitates, however, are darkly colored which indicates there is considerable occlusion of impurities in the precipitates and that oxidation is probably not complete. At the shorter hydrolysis times perhaps one effect will offset another and thus there is no difference in the weight of the precipitates. As the oxidation times were increased the precipitate lost much of its darker color and took on the appearance of a sulphate precipitate. From a previous experiment (Table VI) we would not expect a completely white precipitate at the lower flour : acid ratios used in these studies.

If two or more completely different methods yield the same value for a sample, it is highly probable that the value as reported is correct. It is unlikely that more than one method would respond to different interfering substances in exactly the

Table X

Comparison of various crystalline clays
on siliceous-calcium detergents.
(Clay : soap ratio 1:1)

Clay	Soap	Color of precipitate
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	In all cases
[illegible]	[illegible]	cream to clear
[illegible]	[illegible]	

same manner and to exactly the same extent. Comparisons of different methods for the determination of a single substance in the same sample are important to decide whether a single method gives reliable results.

Determinations of cystine were carried out on the same gluten by the cuprous mercaptide method (19) and the differential-oxidation procedure. In addition gluten sample one was analysed for methionine by the method of Lavine, which is based on the reaction of methionine with iodine, and the differential-oxidation procedure. The results are reported in Table A.

Samples one and two are glutens from Thatcher wheat grown at respectively Fallis and Edmonton.

TABLE A

Cystine and methionine determinations on gluten using different methods

	Cystine Mg. per gm. gluten		Methionine Mg. per gm. gluten	
Sample	Cu. mercaptide method	Differential-oxid.procedure	Lavine method	Differential-oxid.procedure
1	17.86 18.03	17.77 17.57	9.03 9.04	- -
Mean	17.95	17.67	9.04	9.91
2	20.83*	20.59*		

*Single determination

The differences in the cystine contents of the gluten as determined by the two methods is within the 5% error allowed between duplicates. In other words there does not appear to be a

Example	Density	Refractive index	Value of $\frac{n-1}{d}$
1	17.40	17.77	0.02
	18.27	17.37	0.00
Mean	17.83	17.57	0.01
2	20.30	20.17	

significant difference between the results of the two methods. The slightly higher result for the cuprous mercaptide method may be due to the presence of purines.

The results of the methionine determinations check within approximately 10% for this sample of gluten.

There is no reliable specific method for the determination of cystine in materials containing large amounts of carbohydrates. Thus it is very difficult to compare accurately results from specific and the differential-oxidation procedures.

A microbiological method for methionine determinations has been used successfully on organic materials which contain large amounts of carbohydrates. This method (35) was used for comparison.

Methionine determinations were carried out on a one-gram flour sample which was hydrolysed at 15 pounds pressure in 25 ml. of 2 N HCl. The hydrolysate was filtered and neutralized. Triplicate determinations were carried out at two dilutions.

Methionine was also determined on the same flour using the differential-oxidation procedure. The results of the two methods are compared in Table B.

TABLE B

Comparison of two methods for the determination of methionine in flour

Microbiological Method		Differential oxidation procedure
Dilution 1:700	Dilution 1:1000	
Methionine %		Methionine %
0.228	0.251	0.275
0.236	0.244	0.261
0.233	0.265	
Mean 0.232	0.243	0.268

RESULTS

Series I - Marquis flour, 1944

Gerbrandt (19) has carried out analyses for the sulphur-containing amino acids in the gluten washed from these flours. He correlated the cystine and methionine contents of the gluten from 100 grams of flour with loaf volume. His conclusion was that both cystine and methionine so determined were better measures of baking quality than was crude protein in the whole wheat. Gerbrandt suggested that the correlations of the sulphur-containing amino acids with loaf volume were higher than those of crude protein and loaf volume because the former were measured on gluten while the latter is a measure of all nitrogen-containing

TABLE 3

Comparison of two methods for the determination of moisture in flour.

Microbiological Method		Chemical method (Brabender Moisture Tester)
Dilution 1:200	Dilution 1:1000	
		Duplicates %
0.252	0.251	0.270
0.254	0.250	0.271
0.253	0.249	
Mean 0.253	0.250	0.270

REFERENCES

Series I - Strength Flour, 1951.

Department (In) has carried out analyses for the colorimetric determination of moisture in flour. Correlating moisture values in the gluten method from these flours. He correlated the cooking and weighing technique of the 1.5 gm from 100 grams of flour with loaf volume. His conclusions were that both cooking and weighing techniques were inferior measures of baking quality when the pseudo viscosity in the wheat meant. Experiment suggested that the correlations of the colorimetric method with loaf volume were slightly less than those of pseudo pentosin and loaf volume because the former were measured on flours milled in a research of all different varieties

compounds in the whole wheat. The quantity of gluten in a flour is usually directly related to loaf volume. For this reason the quantity of cystine or methionine in gluten from 100 grams of flour could be a better measure of loaf volume than the percentage nitrogen in the wheat. In other words the sulphur-containing amino acids of the gluten in 100 grams of flour are highly correlated with loaf volume because they are direct measures of gluten quantity.

As a method was available for the determination of cystine and methionine in flour it was of interest to compare with the previous work the correlations of loaf volume and the sulphur-containing amino acids in the complete flour.

The sulphur, cystine, methionine and nitrogen contents were determined on the flours and are presented in Table XI. The loaf volumes, which are the same as those used by Gerbrandt, are also included.

For the cystine determinations in this series, the samples were oxidized with 7 ml. of nitric acid per gram flour. Checks between duplicate samples were accepted when the values agreed within 5%.

The calculated correlation coefficients are given in Table XII.

TABLE XI

Data - Marquis flour, 1944

Station	Sulphur %	Cystine %	Methionine %	Nitrogen %	Loaf Volume cc.
Athabaska	0.137	0.385	0.16	1.64	485
Bon Accord	0.170	0.419	0.27	2.47	578
Edmonton (fallow)	0.180	0.453	0.28	2.82	740
Edmonton (stubble)	0.194	0.507	0.27	2.98	857
Fallis	0.144	0.399	0.18	2.04	497
Sundre	0.113	0.305	0.15	1.69	490
Warburg	0.143	0.375	0.20	2.22	548
Composite	0.183	0.491	0.24	2.73	750

TABLE XII

Calculated statistics for Marquis flour, 1944.

r_{VC}	= 0.892**	r_{CS}	= 0.977**	b_{CS}	= 0.230%C/0.1 % S.
$r_{VC.N}$	= 0.385	r_{CN}	= 0.888**	b_{MS}	= 0.17%M/0.1 % S.
r_{VM}	= 0.69*	r_{MS}	= 0.79*		
$r_{VM.N}$	= − 0.24	r_{MN}	= 0.80*		
r_{VS}	= 0.906**	r_{CM}	= 0.69*		
$r_{VS.N}$	= 0.164	r_{SN}	= 0.953**		
r_{VN}	= 0.932**	$r_{CS.M}$	= 0.942**		
$r_{VN.C}$	= 0.675*	$r_{CN.S}$	= − 0.669*		
$r_{VN.M}$	= 0.876**	$r_{MS.N}$	= 0.14		
$r_{VN.S}$	= 0.515	$r_{MN.S}$	= 0.26		

V = loaf volume, cc's

N = nitrogen in flour, %

S = sulphur in flour, %

C = cystine in flour, %

M = methionine in flour, %

* Significant beyond 5% point.

** Significant beyond 1% point.

Table III

Tabulated ephelantes for Bardwell Clone, 1934.

The simple and partial correlation coefficients r_{VN}, $r_{VN.S}$, r_{VS} and $r_{VS.N}$ show that the effect of nitrogen and sulphur contents of flour on loaf volume are apparently related. A relationship is expected because the simple correlation of sulphur and nitrogen is highly significant.

The simple correlations of sulphur and nitrogen content of wheat with loaf volume as described by Gerbrandt are highly significant. These correlations in conjunction with those determined in this study indicate that the sulphur and nitrogen contents of the flour are closely related to the sulphur and nitrogen contents of the wheat.

Nitrogen is a measure of the quantity of crude protein in flour. As the nitrogen and sulphur of the flour are highly significantly correlated there must be a relationship between the sulphur content and the quantity of protein in the flour. The measurement of the sulphur content of flour is therefore another measure of protein quantity and thus there is a high correlation between loaf-volume and sulphur content. However with the effect of nitrogen removed the correlation is not significant, indicating that the effect of sulphur on loaf volume is directly associated with the nitrogen effect.

The cystine, methionine and loaf volume statistics indicate that the quantity of sulphur-containing amino acids in flour is another measure of the quantity of protein in the flour. When the quantity of protein is kept constant there is no significant correlation of cystine or methionine with

loaf-volume. This relationship does not show up in Gerbrandt's study probably because his determinations of the amino acids were on crude gluten while the nitrogen was determined on the whole wheat.

That the cystine and methionine contents of the flour do not affect loaf volume is shown by the values for the partial coefficients $r_{NV.C}$ and $r_{NV.M}$. The nitrogen does affect the loaf volume.

Gerbrandt found that the quantity of cystine and methionine in the gluten in 100 grams of flour were factors which affected loaf volume. The conclusion from the present analysis is that loaf volume is more closely associated with protein content than with the content of the sulphur-containing amino acids in the flour.

As there are only eight samples in this experiment, the values of the simple and partial correlation coefficients indicate that the cystine content of flour is related to the total sulphur but not to the nitrogen content of the flour. The calculated regression line of cystine and sulphur is shown in Figure I,A. The regression coefficient is given in Table XII.

Gerbrandt found a highly significant correlation between the cystine content of gluten and the sulphur content of wheat. As the cystine and sulphur in flour are highly correlated and assuming the sulphur content of wheat and flour is closely

related, a close correlation between the cystine contents of gluten, flour and wheat would be expected.

The methionine, sulphur and nitrogen correlations show that the effects of the nitrogen and sulphur contents of flour on the methionine content are apparently related. These data suggest that nitrogen and sulphur affect the methionine content of flour in much the same way as when taken together. Figure I,B gives the regression line of methionine and sulphur. The regression coefficient is given in Table XII.

The correlation coefficient of cystine and methionine suggests a relationship between the quantities of these amino acids in flour.

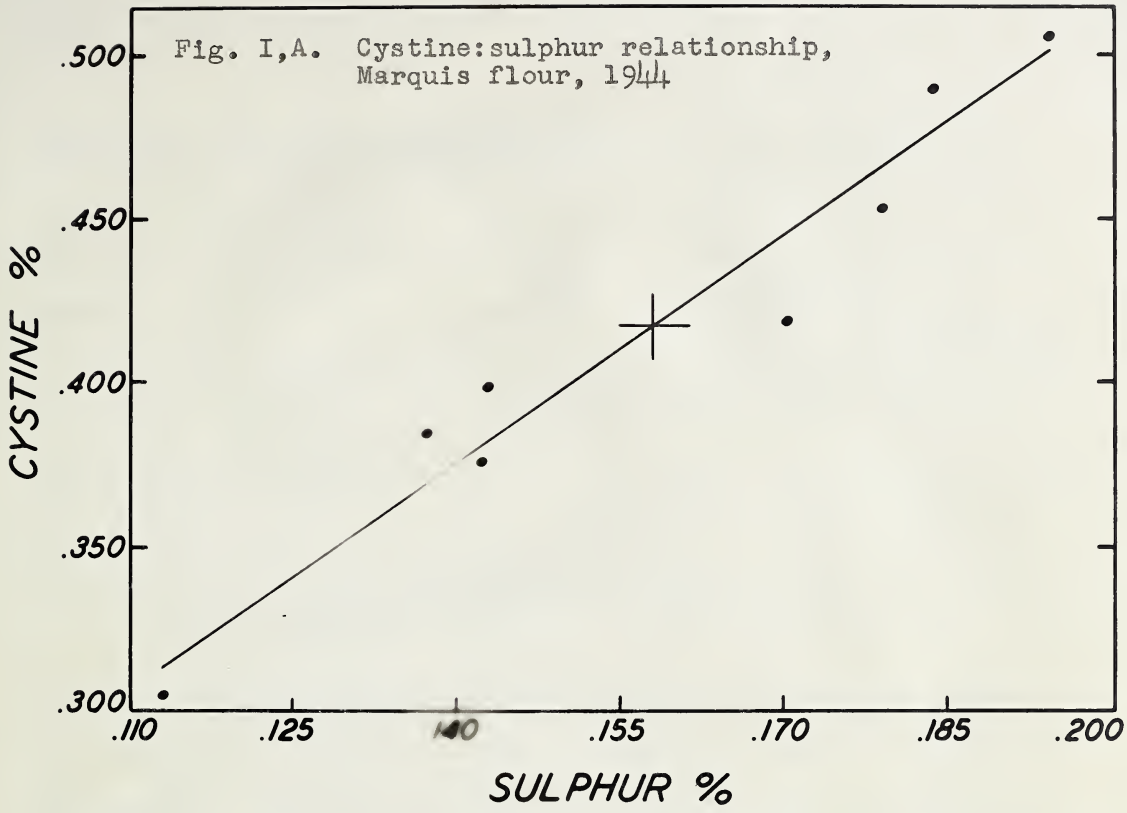


Fig. I,A. Cystine:sulphur relationship, Marquis flour, 1944

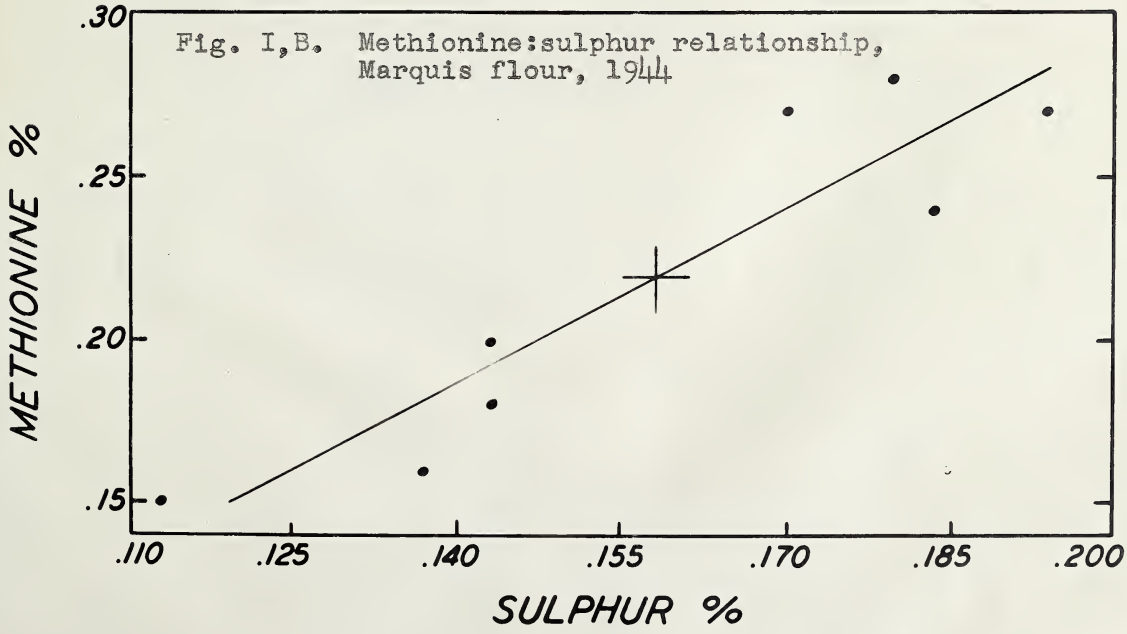


Fig. I,B. Methionine:sulphur relationship, Marquis flour, 1944

Series II - Thatcher wheat and flour, Breton, 1946 and 1947.

The material in this series is valuable for a study of factors affecting the sulphur and sulphur-containing amino acids because of the wide range in sulphur content.

Loaf volume relationships were determined only for the 1947 crop because, as was previously stated, there are very small volume differences in the 1946 crop. With a baking error of 60 cc. few plots in either Series E or B of the 1946 crop produced loaf volumes significantly different from the mean.

Table XIII and Figure II, A, B, C and D show the sulphur, cystine, methionine and nitrogen contents of wheat harvested from the continuous wheat plots of the Breton field. Included in the table are the loaf volumes and cystine-sulphur as a percentage of the total sulphur.

The analyses of variance for sulphur, cystine, methionine and nitrogen are given in Table XIV.

The variations in the nitrogen content of the wheat in the continuous rotation are very small. This is shown graphically in Figure II, C. The application of fertilizers had little affect on the nitrogen content of the wheat.

TABLE XIII

Data - Thatcher wheat, Series E,
Breton, 1946.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Loaf Volume	% Cystine-sulphur of total sulphur
1	0.132	0.349	0.18	1.86	418	71
2	0.113	0.358	0.08	2.09	475	84
3	0.125	0.378	0.11	1.77	445	81
4	0.126	0.388	0.10	1.76	428	82
5	0.130	0.315	0.21	1.85	418	65
6	0.127	0.372	0.13	1.82	435	78
7	0.127	0.355	0.15	2.03	435	74
8	0.146	0.404	0.18	2.19	465	74
9	0.146	0.448	0.12	1.94	475	82
10	0.126	0.385	0.11	2.15	435	82
11	0.118	0.338	0.13	2.07	428	76
12	0.173	0.518	0.16	2.15	475	81
13	0.162	0.525	0.10	2.06	455	87
14	0.136	0.398	0.14	1.93	408	78
15	0.162	0.495	0.14	2.03	505	82
16	0.135	0.391	0.14	1.81	445	78
17	0.157	0.454	0.17	2.02	465	77
Mean	0.139	0.404	0.14	1.97	448	78

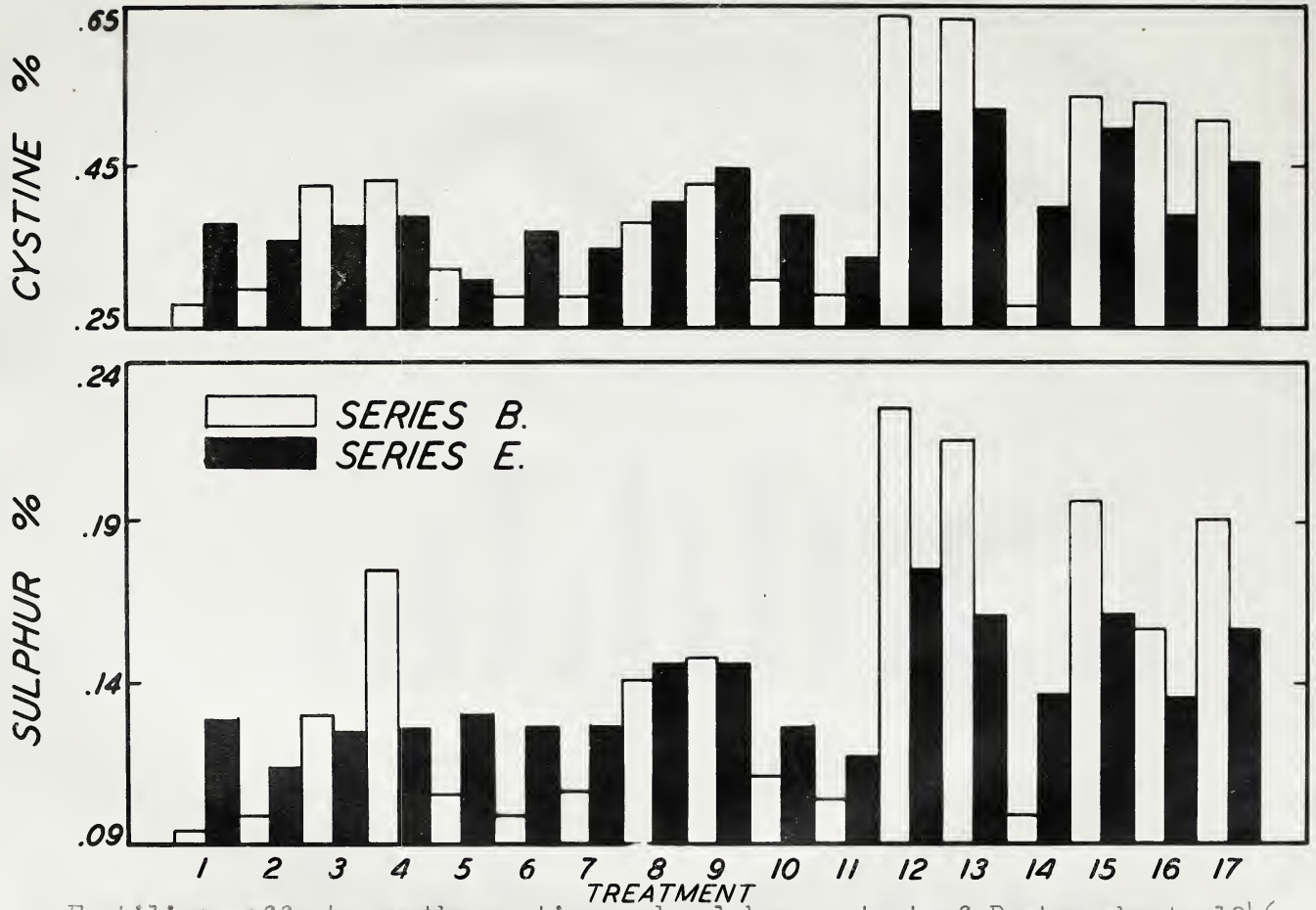
Table III

Data - Nitrogen wheat, Garden B,
Season, 1924.

Sample No.	Sulphur %	Cyanides %	Sulphides %	Nitrogen %	Loss Volume	Percentage of burnt sulphur
1	0.152	0.017	0.13	1.86	112	71
2	0.115	0.150	0.20	2.09	120	61
3	0.130	0.07	0.11	1.77	120	67
4	0.128	0.08	0.10	1.76	120	72
5	0.130	0.12	0.21	1.8	120	72
6	0.127	0.172	0.12	1.8	120	67
7	0.127	0.16	0.1	2.03	120	70
8	0.130	0.10	0.12	2.13	120	72
9	0.128	0.10	0.1	1.94	120	72
10	0.128	0.08	0.11	2.15	120	72
11	0.112	0.16	0.13	2.07	120	72
12	0.127	0.11	0.16	2.16	125	71
13	0.142	0.125	0.10	2.36	120	72
14	0.130	0.09	0.11	1.79	120	70
15	0.132	0.08	0.11	2.12	105	72
16	0.135	0.102	0.14	1.93	105	70
17	0.127	0.084	0.17	2.09	105	77
Mean	0.129	0.104	0.13	1.97	114	72

Fig. II,A.

Fig. II,B.



Fertilizer effects on the cystine and sulphur content of Breton wheat, 1946.

Fertilizer effects on the nitrogen and methionine content of Breton wheat, 1946.

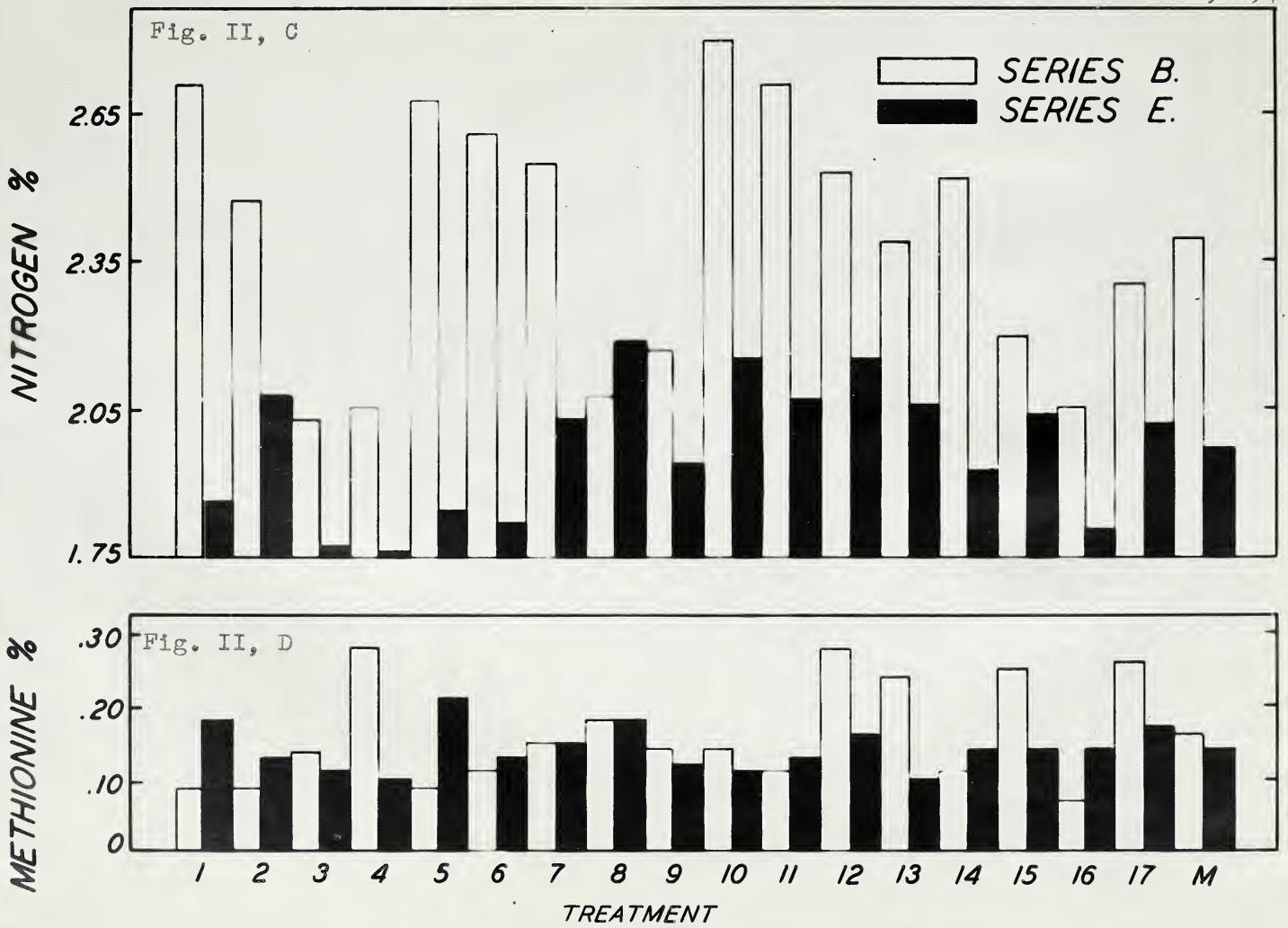


TABLE XIV

Analysis of Variance - Series E Wheat.

Variance due to	D.F.	Mean Square Nitrogen	Mean Square Sulphur	Mean Square Cystine	Mean Square Methionine
Treatment	16	0.03900**	0.0005884**	0.007839**	0.002243**
Error	17	0.00070	0.0000149	0.000045	0.000665
Total	33				

**Significant beyond the 1% point.

Minimum significant differences for treatment are:

0.008% sulphur.

0.05% nitrogen.

0.013% cystine.

0.05% methionine.

In most cases the application of sulphur fertilizers increases the sulphur content of the wheat. Usually the sulphur fertilized plots produce wheat significantly higher in cystine content than the non-fertilized plots. As would be expected the cystine content of the wheat is closely related to the sulphur content. As a rule the high sulphur wheats are highest in cystine content while the low sulphur wheats are lowest. The correlation coefficients show the close relationship between the cystine and sulphur contents of this wheat.

The calculated correlation and regression coefficients are presented in Table XV. The cystine, sulphur scatter is

plotted in Figure III, A.

The correlations and bar diagrams show that the cystine content of wheat is influenced more by the sulphur than the nitrogen content.

An average of 78 percent of the total sulphur in the wheat of this series is present as cystine-sulphur. There does not appear to be any relationship between the quality or quantity of fertilizers applied and the percentage of total sulphur represented as cystine-sulphur.

There are very small if any variations in the methionine contents of the various wheats from Series E. Check plot one is reported as containing 0.18 per cent methionine. This value appears relatively high. An error in either the cystine or sulphur determination would give such a result. Assuming either the cystine or the sulphur percentage as correct it is possible to obtain the alternate constituent from the regression line in Figure III, A or from the regression coefficient in Table XV. A corrected methionine content can be calculated as a difference between the cystine-sulphur and the total sulphur values so obtained. The corrected value for the check plot one is 0.15%. Using this value as a check, the wheat from the lime plot is significantly higher and the wheat from the manure plot significantly lower in methionine. The sulphur and cystine contents of these wheats suggest that the values are misrepresented due to errors in the determinations. When the values for methionine in these wheats are corrected as in the case of the check plot,

there are no significant differences in the methionine of the wheat from Series E.

The results suggest that the methionine content of the continuous wheat is not affected by the applications of fertilizers.

The correlation coefficients r_{MS} and $r_{MS.N}$ suggest that the methionine in wheat is not influenced by the sulphur content. This is not what we would expect. In this particular series the methionine range is very small and a few values considerably out of line would upset the methionine-sulphur correlation.

The scatter diagram of methionine and sulphur in Figure III, B shows how a few values will destroy the correlation. When the values obviously out of line are corrected by the use of the cystine, total sulphur regression, the methionine content is directly related to the sulphur content of the wheat. There is no relationship between the methionine and nitrogen contents of wheat.

The percentages of the total sulphur present in wheat as methionine-sulphur can be obtained from Table XIII. Obviously the values are the differences between the cystine percentages and 100 percent.

TABLE XV

Regression and Correlation Coefficients, Series E, Continuous Wheat, Breton, 1946.

r_{CS}	= 0.908**	b_{CS}	= 0.332%C/0.1%S.
$r_{CS.N}$	= 0.951**	b_{MS}	= 0.06%m./0.1%S.
r_{CN}	= 0.369		
$r_{CN.S}$	= 0.130		
r_{MS}	= 0.28		
$r_{MS.N}$	= 0.29		
r_{MN}	= 0.01		
$r_{MN.S}$	= − 0.09		
r_{CM}	= − 0.15		
r_{SN}	= 0.350		

C = % cystine in flour

S = % sulphur in flour

N = % nitrogen in flour

M = % methionine in flour

* Significant beyond the 5% point.

** Significant beyond the 1% point.

Figure III - A - Relation between cystine and sulphur.
Series E wheat, Breton, 1946.

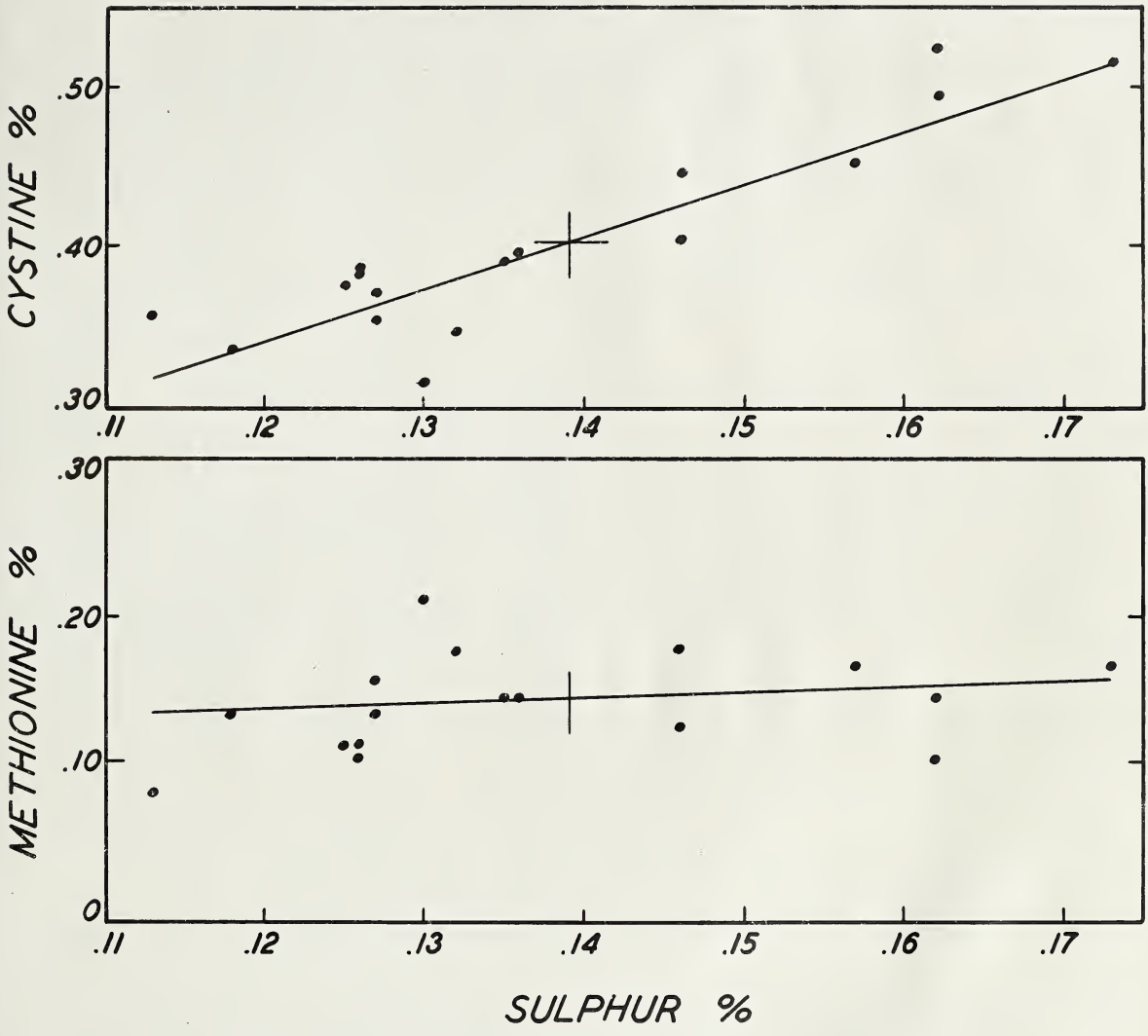


Figure III, B - Relation between methionine and sulphur.
Series E wheat, Breton, 1946.

The sulphur, cystine, methionine and nitrogen contents of the wheat grown on Series B, Breton plots are presented in Table XVI and Figure II. The loaf volumes and the values for cystine-sulphur as a percentage of the total sulphur in the wheat are also presented in the table.

The analyses of variance for sulphur, cystine, methionine and nitrogen are given in Table XVII.

The calculated correlation and regression coefficients are presented in Table XVIII.

The loaf volumes of Series B exhibit a slightly greater range than those of Series E. No attempts were made to correlate loaf volume with the other results because the range of volumes is within the 60 cc. baking error of the mean.

The diagrams in figure II show that generally speaking wheat after clover has a greater range for each constituent determined than the continuous wheat.

The sulphur-containing fertilizers have decreased the nitrogen content of the wheat more significantly than the others. The lower nitrogen content of the grain can be attributed to increased yields resulting from the application of sulphur fertilizers (41). Table XVI and figure II, A show that the application of fertilizers containing significant amounts of sulphur have resulted in increases in the sulphur content of wheat. The increases in sulphur and decreases in nitrogen caused by the application of sulphur fertilizers explain the negative and significant simple correlation coefficient for sulphur and nitrogen.

TABLE XVI

Data - Thatcher Wheat, Series B,
Breton, 1946.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Loaf Volume	Cystine-sulphur as % of total sulphur
1	0.094	0.283	0.09	2.71	485	80.3
2	0.099	0.298	0.09	2.48	495	80.3
3	0.145	0.429	0.14	2.03	435	79.0
4	0.177	0.434	0.28	2.06	425	65.4
5	0.105	0.324	0.09	2.68	485	82.3
6	0.099	0.284	0.11	2.61	465	76.5
7	0.107	0.282	0.15	2.55	505	70.3
8	0.141	0.380	0.18	2.08	438	71.9
9	0.148	0.442	0.14	2.17	418	79.7
10	0.111	0.304	0.14	2.80	563	73.1
11	0.103	0.294	0.11	2.71	495	76.1
12	0.232	0.642	0.28	2.53	553	73.8
13	0.220	0.635	0.24	2.39	553	77.0
14	0.098	0.279	0.11	2.52	495	75.9
15	0.197	0.535	0.25	2.20	553	72.4
16	0.157	0.529	0.07	2.05	495	89.9
17	0.191	0.504	0.26	2.31	465	70.4
Mean	0.143	0.405	0.16	2.40	490	75.6

Sample No.	Sulphur %	Organic %	Nobleniums %	Nitrogen %	Total Volume	Organic sulphur as % of total sulphur
1	0.094	0.092	0.00	0.73	[illegible]	97.9
2	0.099	0.098	0.00	0.76	[illegible]	98.9
3	0.116	0.114	0.11	0.79	[illegible]	98.2
4	0.117	0.115	0.20	0.85	[illegible]	98.3
5	0.105	0.103	0.09	0.80	[illegible]	98.1
6	0.095	0.093	0.11	0.82	[illegible]	97.9
7	0.097	0.095	0.12	0.83	[illegible]	97.9
8	0.101	0.099	0.10	0.80	[illegible]	98.0
9	0.106	0.104	0.10	0.82	[illegible]	98.1
10	0.111	0.109	0.11	0.86	[illegible]	98.2
11	0.102	0.100	0.11	0.81	[illegible]	98.0
12	0.093	0.091	0.09	0.78	[illegible]	97.8
13	0.089	0.087	0.08	0.75	[illegible]	97.8
14	0.098	0.096	0.11	0.79	[illegible]	98.0
15	0.097	0.095	0.09	0.77	[illegible]	97.9
16	0.107	0.105	0.07	0.83	[illegible]	98.1
17	0.102	0.100	0.08	0.81	[illegible]	98.0
Mean	0.103	0.101	0.11	0.80	[illegible]	98.0

TABLE XVII

Analysis of Variance, Series B,
Breton Wheat, 1946.

Variance due to	D.F.	Sulphur Mean Square	Cystine Mean Square	Methionine Mean Square	Nitrogen Mean Square
Treatment	16	0.004265**	0.031931**	0.01085**	0.14379**
Error	17	0.0000186	0.0000982	0.0009824	0.00151
Total	33				

**Significant beyond the 1% point.

Minimum significant differences for treatment are: 0.009% sulphur,
0.020% cystine,
0.06% methionine,
0.08% nitrogen.

TABLE XVIII

Regression and Correlation Coefficients, Series B,
Wheat after Clover, 1946.

$r_{CS.} = 0.968^{**}$ $r_{MS.N} = 0.83^{**}$ $b_{CS} = 0.265\%C/0.1\%S.$

$r_{CS.N} = 0.957^{**}$ $r_{MN} = -0.34$ $b_{MS} = 0.13\%M/0.1\%S.$

$r_{CN} = -0.510^{*}$ $r_{MN.S} = 0.18$

$r_{CN.S} = -0.138$ $r_{MC} = 0.69^{**}$

$r_{MS} = 0.85^{**}$ $r_{SN} = -0.496^{*}$

*Significant beyond 5% point
**Significant beyond 1% point.

C = % cystine in flour
M = % methionine in flour
S = % sulphur in flour
N = % nitrogen in flour

TABLE XVII

Analysis of Variance, Spring 1.
Bruton Sweet, 1936.

Variance due to	D.F.	Mean Square	Spring Mean Square	Residual Mean Square	Average Mean Square
Treatment	16	0.0052852**	0.0431934**	0.0105****	0.1479**
Error	17	0.0000760	0.0000820	0.0000722	0.0761
Total	33				

** Significant beyond the 1% point.

Minimum significant differences for treatment pre: 0.0092 ordpre.
0.0259 cropline.
0.008 mercklenline.
0.007 nicrotope.

TABLE XVIII

Regression and Correlation Coefficients, Series B.
Wheat after Clover, 1936.

$r_{ds.}$ = 0.968**	$r_{MS.N}$ = 0.[illegible]	r_{Ca} = 0.166**/0.714.
$r_{Ca.N}$ = 0.957**	r_{MK} = −0.24	r_{Ca} = 0.134**/0.184.
r_{Cy} = −0.910*	$r_{MN.S}$ = 0.10	
$r_{CN.S}$ = −0.122	r_{Mo} = 0.09**	
r_{Bo} = 0.85**	r_{SN} = −0.106*	

** Significant beyond 1% point.
* Significant beyond 5% point.

The effect of the fertilizers on the cystine content of the wheat is similar to the effect on the sulphur content. This situation is similar to that observed in the wheat after wheat series. The regression line for cystine and sulphur is plotted in figure IV,A. As in the wheat after wheat series, the cystine content of Series B wheat is not related to the nitrogen content but is closely associated with the sulphur content. The application to wheat of fertilizers containing large amounts of sulphur results in large and significant increases in cystine and sulphur contents. The fertilizer treatments decrease the nitrogen content of wheat but this apparently has no effect on the cystine content.

Approximately 70 to 80 percent of the sulphur in Series B wheat is present as cystine-sulphur. This is a similar proportion to that in the Series E wheat. There is no apparent relationship between fertilizer treatment and the amount of sulphur present in cystine.

Generally the application of sulphur-containing fertilizers has resulted in increases in the methionine content of the wheat. The regression line figure IV, B shows that the methionine and sulphur contents of wheat are closely related. Nitrogen variations in the wheat have no influence on the methionine content.

The simple correlation coefficient r_{MC} is positive and highly significant. This suggests that in the wheat proteins of Series B the methionine and cystine contents are related.

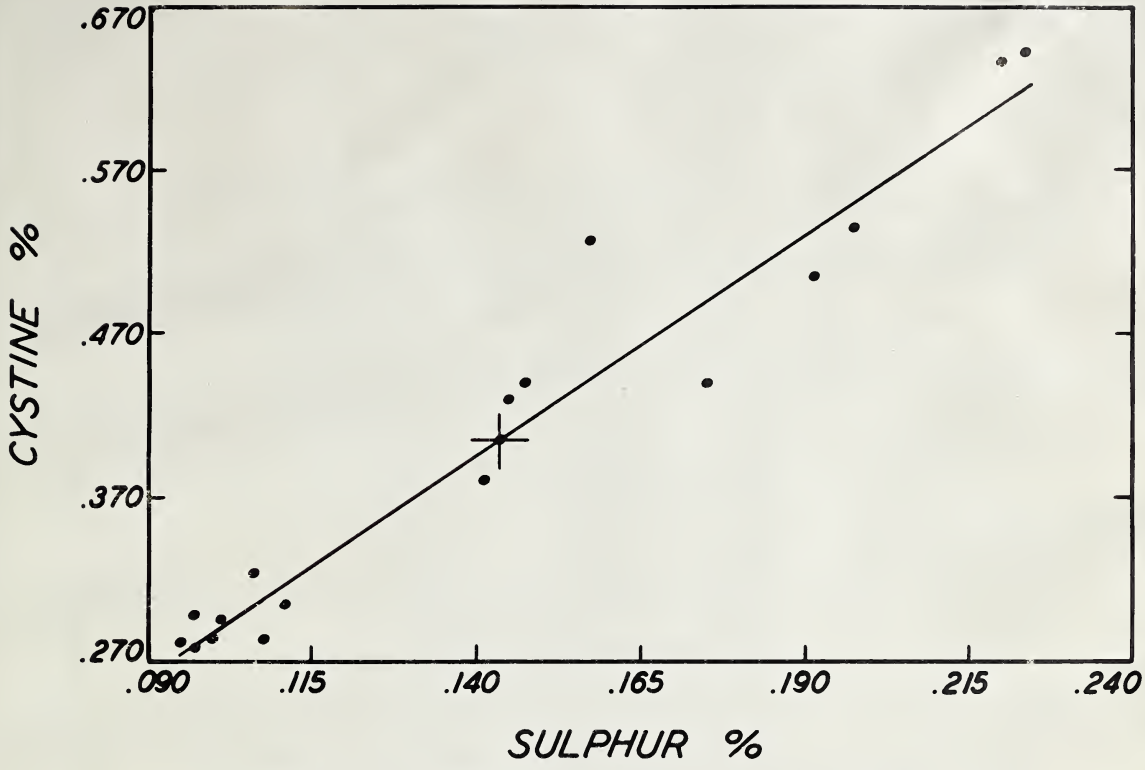


Figure IV, A - Cystine:sulphur relationship,
Breton wheat, Series B.

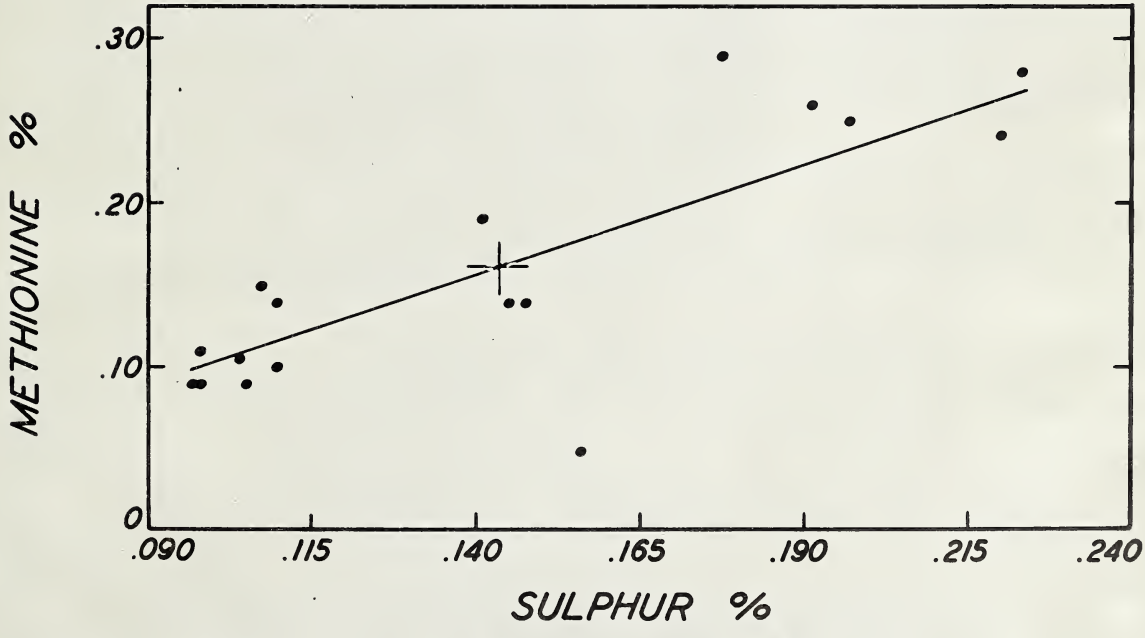


Figure IV, B - Methionine:sulphur relationship,
Breton wheat, Series B.

The analyses of flours from Series E and B were carried out to determine whether the sulphur, cystine, methionine and nitrogen relationships in flour are the same as in wheat.

The sulphur, cystine, methionine and nitrogen contents of the flours from Series E wheat are presented in Table XIX. The percentages as reported are slightly lower in the flour than in the wheat. There is a close relationship between these constituents in wheat and flour. This can be readily seen from the correlation coefficients in Table XXI. The wheat samples with the higher sulphur, cystine, methionine and nitrogen values produce flour higher in these constituents. The lower values for the wheat and flour are also related.

The analyses of variance for the data are given in Table XX. Fertilizer treatments have resulted in significant differences for the constituents determined.

The correlation and regression coefficients for Series E are presented in Table XXI. The regression lines for cystine and sulphur and methionine and sulphur are plotted in figure V, A and B. These data show that the cystine and methionine contents of flour, as of wheat, are determined by sulphur rather than nitrogen content of the flour.

The cystine-sulphur as a percentage of the total sulphur in the flour is included in Table XIX.

The significant simple correlation coefficient, r_{MC} suggests that the methionine and cystine in the flour are related. This was not observed in Series E wheat.

TABLE XIX

Data - Flour from Thatcher Wheat,
Series E, 1946.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Cystine-sulphur as % of total sulphur
1	0.114	0.317	0.14	1.79	74
2	0.120	0.322	0.16	1.98	72
3	0.128	0.329	0.19	1.65	69
4	0.116	0.341	0.12	1.59	78
5	0.122	0.337	0.15	1.84	74
6	0.116	0.299	0.17	1.84	69
7	0.114	0.325	0.13	1.82	76
8	0.144	0.404	0.17	2.04	75
9	0.143	0.382	0.19	2.05	71
10	0.129	0.365	0.15	1.94	76
11	0.109	0.320	0.11	1.99	78
12	0.161	0.448	0.19	2.04	74
13	0.160	0.441	0.19	1.98	74
14	0.131	0.352	0.17	1.93	72
15	0.163	0.429	0.23	1.95	70
16	0.127	0.376	0.12	1.74	79
17	0.123	0.367	0.12	1.79	80
Mean	0.131	0.362	0.16	1.88	74

Table 4.11

Table - Major Cross Tabulator Output.
Series A, Table 1.

Case No.	Malaise %	Cyanosis %	Debilitation %	Allergies %	Diagnosis - Allergy as % of Total Malaise
1	0.114	0.117	0.0	1.73	1%
2	0.129	0.322	0.10	1.38	2
3	0.138	0.129	0.20	1.65	2
4	0.116	0.141	0.15	1.22	2
5	0.142	0.277	0.25	1.31	2
6	0.136	0.219	0.17	1.64	2
7	0.114	0.129	0.15	1.62	2
8	0.146	0.084	0.17	2.04	2
9	0.145	0.482	0.17	1.05	2
10	0.129	0.295	0.13	1.64	2
11	0.109	0.229	0.11	1.44	2
12	0.141	0.084	0.13	1.06	2
13	0.136	0.142	0.13	1.22	2
14	0.134	0.134	0.11	1.22	2
15	0.143	0.229	0.11	1.04	2
16	0.127	0.276	0.15	1.34	2
17	0.129	0.307	0.12	1.20	2
Mean	0.131	0.202	0.14	1.38	2%

TABLE XX

Analysis of Variance, Series E flour,
Breton, 1946.

Variance due to	D.F.	Mean Squares			
		Sulphur	Cystine	Methionine	Nitrogen
Treatment	16	0.000603**	0.004203**	0.00225*	0.03864**
Error	17	0.0000153	0.0000512	0.000712	0.000529
Total	33				

* Significant beyond 5% point.
** Significant beyond 1% point.

The minimum significant differences for treatment are:

0.008% sulphur

0.014% cystine

0.05% methionine

0.05% nitrogen.

Table XX

Analysis of Variance, Series A Tissue,
Oxygen, 10%.

Variance due to	D.F.	Sum of Squares	Mean Square	Variance Ratio	Significance
Treatment	10	0.0000[illegible]	0.0000[illegible]	0.0023*	0.05[illegible]
Error	17	0.0000[illegible]	0.0000[illegible]	0.0017[illegible]	0.0[illegible]
Total	27				

* significant beyond 5% point.
** Significant beyond 1% point.

The minimum significant differences for treatment are:

0.00[illegible] nitrogen.

0.00[illegible] methionine

0.00[illegible] lysine

0.00[illegible] tryptophan

TABLE XXI

Calculated statistics for Series E flour, 1946.

r_{CS}	= 0.946**	r_{MC}	= 0.56*
$r_{CS.N}$	= 0.930**	r_{SN}	= 0.530*
$r_{CN.}$	= 0.483	r_{WN}	= 0.604**
$r_{CN.S}$	= −0.066	r_{SH}	= 0.845**
r_{MS}	= 0.80**	b_{CS}	= 0.246%C./0.1%S.
$r_{MS.N}$	= 0.75**	b_{CM}	= 0.08%M./0.1%S.
r_{MN}	= 0.41		
$r_{MN.S}$	= −0.20		

* Significant beyond 5% point.
** Significant beyond 1% point.

S = % sulphur in flour

N = % nitrogen in flour

C = % cystine in flour

M = % methionine in flour

W = % nitrogen in wheat

H = % sulphur in wheat.

Series E flour, Breton, 1946

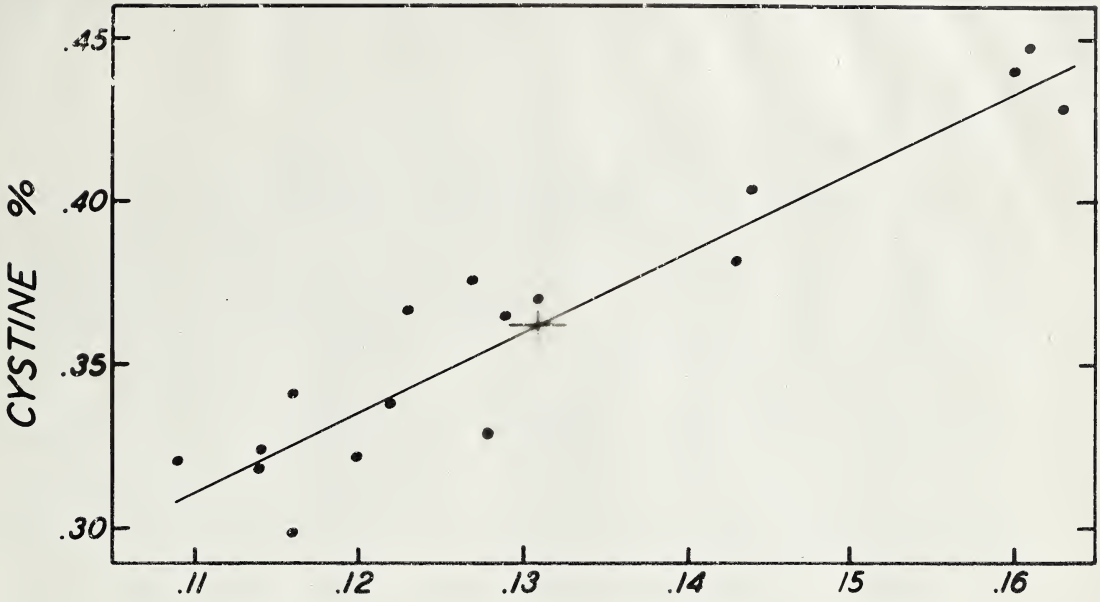


Figure V, A - Cystine:sulphur relationship.

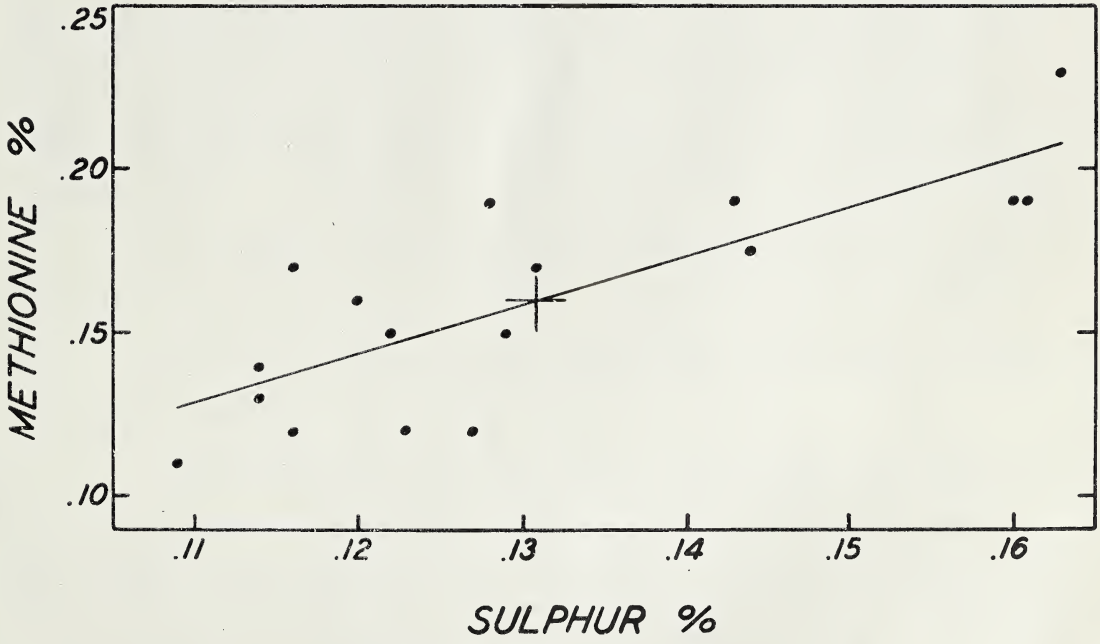


Figure V, B - Methionine:sulphur relationship.

The sulphur, cystine, methionine and nitrogen contents of the flour milled from Series B wheat are presented in Table XXII. The cystine-sulphur as a percentage of the total sulphur is included in the table.

Generally there is a close relationship between the quantities of determined constituents in the flour and wheat. This is shown by the correlation coefficients in Table XXIV. The sulphur and cystine contents are generally smaller in the flour than in the wheat. The methionine values for flour, on the other hand, are not consistent. They are sometimes higher and sometimes lower than the values for wheat. Nitrogen determinations were not carried out on all of the Series B flour because of insufficient material. There is, however, a trend towards a lower nitrogen content in the flour than in the wheat.

The results of the simple analyses of variance on the data are presented in Table XXIII. The fertilizer treatments have a significant effect on the quantities of sulphur, cystine, methionine and nitrogen in the flour.

The correlation and regression coefficients for Series B are presented in Table XXIV. The regression lines for cystine and sulphur and methionine and sulphur are plotted in Figure VI, A and B. The relationships between sulphur, cystine, methionine and nitrogen are similar to those observed in Series E flour. The sulphur-containing amino acid content of flour is determined by sulphur rather than nitrogen content of flour.

TABLE XXII

Data - Flour from Thatcher Wheat,
Series B, 1946.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Cystine-Sulphur as % of Total Sulphur.
1	0.105	0.326	0.09	-	83
2	0.084	0.266	0.06	2.37	85
3	0.151	0.419	0.18	-	74
4	0.159	0.342	0.32	-	57
5	0.118	0.210	0.28	2.55*	48
6	0.083	0.273	0.05	2.52	88
7	0.097	0.284	0.10	2.39	78
8	0.133	0.361	0.17	2.06	72
9	0.144	0.436	0.13	2.05	81
10	0.102	0.269	0.14	2.66*	70
11	0.107	0.273	0.16	-	68
12	0.214	0.571	0.29	2.51	71
13	0.220	0.608	0.32	2.43	74
14	0.115	0.385	0.06	2.54*	89
15	0.190	0.488	0.28	2.21	69
16	0.145	0.493	0.13	2.13	82
17	0.184	0.530	0.20	2.32*	77
Mean	0.138	0.381	0.18	2.36	74

*Single determination

Sample No.	Moisture %	Organic Matter %	Available P	Nitrogen %	Water pH Unit
1	0.109	0.329	0.09	-	6
2	0.068	0.2[illegible]	0.08	0.07	6
3	0.171	0.103	0.21	-	6
4	0.151	0.[illegible]62	0.[illegible]6	-	6
5	0.115	0.234	0.[illegible]5	0.[illegible]4	6
6	0.[illegible]7	0.[illegible]7[illegible]	0.[illegible]6	0.[illegible]3	6
7	0.[illegible]4	0.[illegible]0	0.[illegible]3	0.[illegible]3	6
8	3.[illegible]3	0.[illegible]61	0.[illegible]7	0.[illegible]3	7
9	0.[illegible]1	0.[illegible]6	0.[illegible]3	0.[illegible]3	6
10	0.[illegible]3	0.[illegible]0	0.[illegible]4	0.[illegible]2	6
11	0.[illegible]7	0.[illegible]5	0.[illegible]5	-	6
12	0.[illegible]1	0.[illegible]1	0.[illegible]3	0.[illegible]1	7
13	0.[illegible]0	0.[illegible]6	0.[illegible]3	0.[illegible]3	6
14	0.[illegible]9	0.[illegible]5	0.[illegible]3	0.[illegible]3	6
15	0.[illegible]9	0.[illegible]5	0.[illegible]3	0.[illegible]3	6
16	0.[illegible]0	0.[illegible]8	0.[illegible]3	0.[illegible]1	6
17	0.[illegible]2	0.[illegible]7	0.[illegible]3	0.[illegible]3	6
18	0.[illegible]3	0.[illegible]7	0.[illegible]3	0.[illegible]3	6
19	0.[illegible]4	0.[illegible]0	0.[illegible]3	0.[illegible]3	6
20	0.[illegible]3	0.[illegible]0	0.[illegible]3	0.[illegible]3	6
Mean	0.[illegible]0	0.[illegible]1	0.[illegible]6	0.[illegible]3	6

*Single determination

TABLE XXIII

Analysis of Variance
Flour from Thatcher Wheat, Series B, 1946.

Variance due to	D.F.	Mean Squares			
		Sulphur	Cystine	Methionine	Nitrogen
Treatment	16	0.003754**	0.02786**	0.01628**	0.06971**
Error	17	0.0000241	0.0000549	0.00136	0.000556
Total	33				

**Significant beyond 1% point.

The minimum significant differences for treatment are:

0.010% sulphur

0.015% cystine

0.07% methionine

0.05% nitrogen.

Variance due to	d.f.	Sum Squares	Mean Squares	F. ratio
Treatment	16	0.0037480	0.0002342	6.8**
Error	17	0.0005841	0.0000344	
Total	33			

**significant beyond 1% point.

TABLE XXIV

Calculated Statistics for Series B flour, 1946.

r_{CS}	= 0.916**	r_{MC}	= 0.47*
$r_{CS.N}$	= 0.914**	r_{SN}	= −0.210
r_{CN}	= −0.291	r_{SH}	= 0.971**
$r_{CN.S}$	= −0.253	b_{CS}	= 0.249%C./0.1%S.
r_{MS}	= 0.78**	b_{CM}	= 0.17%M./0.1%S.
$r_{MS.N}$	= 0.80**		
r_{MN}	= 0.02		
$r_{MN.S}$	= 0.29		

*Significant beyond 5% point.
**Significant beyond 1% point.

S = % sulphur in flour

C = % cystine in flour

N = % nitrogen in flour

M = % methionine in flour

H = % sulphur in wheat.

Series B flour, Breton, 1946.

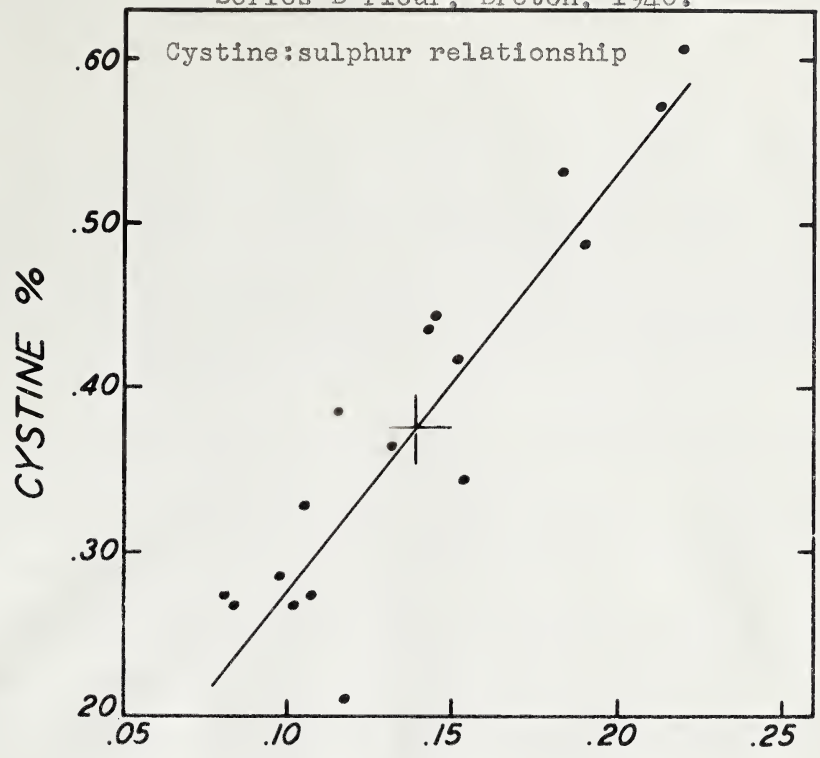


Fig. VI,A

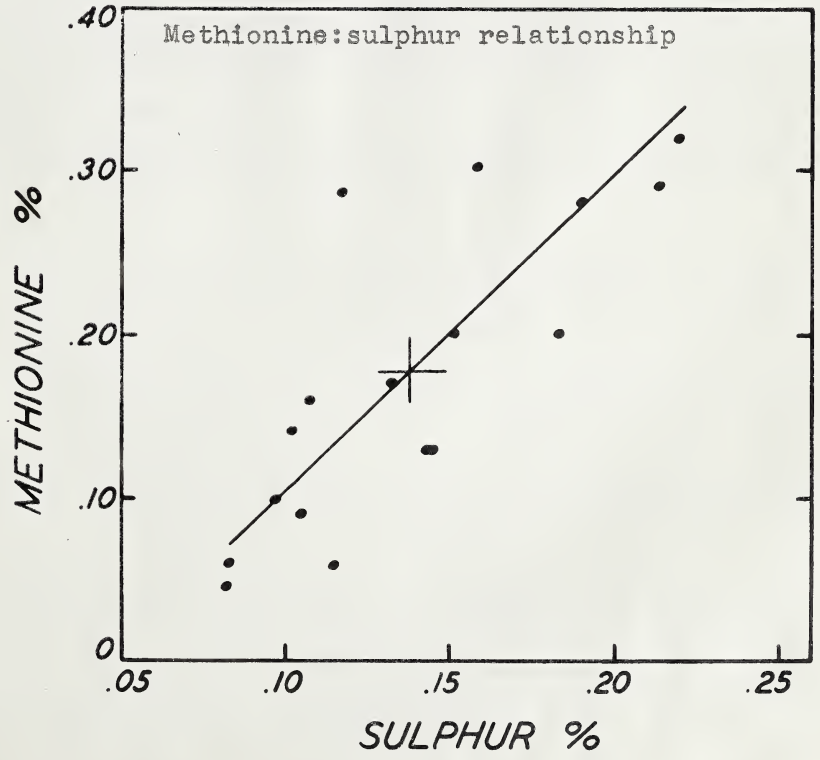


Fig. VI,B

When wheat is milled there are considerable quantities of bran, feed flour and red dog flour removed. From the available data an estimate can be made of the percentage of the total cystine and methionine in wheat which remains in the flour.

Table XXV gives the amounts of cystine and methionine in the flour as the percentage of the sulphur-containing amino acids present in the wheat. The percentage extraction of flour from wheat varies for each individual sample. The exact extraction data were not available. For the calculations reported it was assumed that the Allis-Chalmers experimental mill extracted 65% of the wheat as flour.

The percentage extraction of cystine in the flour is approximately the same in both series. These data show that approximately 60% of the cystine in wheat is extracted with the flour.

The values for the methionine contents of wheat or flour of sample 16, Series B and samples 2, 3, 9, 13 and 15, Series E are not accurate. This is obvious because more than 100% extraction is obtained. Table XXV shows that approximately 60% of the methionine in the wheat of both series is extracted in the flour. The mean and standard deviation for Series E are considerably greater than the values for Series B. If the values in Series E above 100% are discarded, the mean and standard deviation are $65 \pm 13\%$.

TABLE XXV

The percentages of cystine and methionine of wheat extracted in the flour of Series E and B

Sample No.	Series E		Series B	
	Cystine %	Methionine %	Cystine %	Methionine %
1	59	51	75	66
2	58	130	58	43
3	57	113	63	84
4	57	78	51	74
5	70	47	42	48
6	52	85	62	30
7	59	57	66	43
8	65	62	62	62
9	55	103	64	61
10	62	89	58	65
11	62	55	60	95
12	56	78	58	68
13	55	124	62	87
14	58	79	90	35
15	56	107	59	73
16	62	56	54	121
17	53	46	68	50
Mean	59 ± 4	80 ± 27	62 ± 10	65 ± 23

The 1947 crop of Thatcher wheat grown in a clover rotation at the Breton station produced bread with large differences in loaf volume. This material was therefore satisfactory for use in a study of loaf volume as affected by the sulphur-containing amino acids.

The sulphur, cystine, methionine and nitrogen were determined in the wheat. The results are presented in Table XXVI and figure VII, A, B, C and D. Included in the table are the loaf volumes and the cystine-sulphur as a percentage of the total sulphur.

[illegible]

[illegible]

Sample No.	Group 1	Group 2	Group 3	Group 4
1	[illegible]	[illegible]	[illegible]	[illegible]
2	[illegible]	[illegible]	[illegible]	[illegible]
3	[illegible]	[illegible]	[illegible]	[illegible]
4	[illegible]	[illegible]	[illegible]	[illegible]
5	[illegible]	[illegible]	[illegible]	[illegible]
6	[illegible]	[illegible]	[illegible]	[illegible]
7	[illegible]	[illegible]	[illegible]	[illegible]
8	[illegible]	[illegible]	[illegible]	[illegible]
9	[illegible]	[illegible]	[illegible]	[illegible]
10	[illegible]	[illegible]	[illegible]	[illegible]
11	[illegible]	[illegible]	[illegible]	[illegible]
12	[illegible]	[illegible]	[illegible]	[illegible]
13	[illegible]	[illegible]	[illegible]	[illegible]
14	[illegible]	[illegible]	[illegible]	[illegible]
15	[illegible]	[illegible]	[illegible]	[illegible]
16	[illegible]	[illegible]	[illegible]	[illegible]
17	[illegible]	[illegible]	[illegible]	[illegible]
Mean	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

[illegible]

[illegible]

Simple analyses of variance of the sulphur, cystine, methionine and nitrogen data are given in Table XXVII. The analyses show that the fertilizer treatments result in significant increases in the sulphur, amino acid and nitrogen contents of Series C wheat.

The calculated correlation and regression coefficients are tabulated in Table XXVIII.

As in the material previously investigated the sulphur content of the wheat increases with the application of sulphur fertilizers. Generally, the cystine contents of the seventeen samples of wheat follow the sulphur contents. The highest sulphur samples have the most cystine, the lowest sulphur samples the least. The relationship between cystine and sulphur is shown graphically in figure VII, A and B and figure VIII, A. The highly significant simple and partial correlation coefficients r_{CS} and $r_{CS.N}$ confirm the close relationship between cystine and sulphur.

TABLE XXVI

Data - Thatcher wheat, Series C (wheat after clover), Breton, 1947.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Loaf Volume	Cystine-sulphur as % of total sulphur
1	0.107	0.286	0.14	2.76	593	71
2	0.121	0.348	0.13	2.95	683	77
3	0.150	0.435	0.16	2.49	645	77
4	0.179	0.502	0.21	2.95	505	75
5	0.111	0.314	0.13	2.83	583	75
6	0.113	0.324	0.12	2.88	673	76
7	0.117	0.262	0.22	2.90	655	60
8	0.132	0.327	0.21	2.80	645	66
9	0.138	0.412	0.13	2.90	630	80
10	0.126	0.371	0.13	2.82	593	79
11	0.149	0.339	0.27	2.71	703	61
12	0.206	0.580	0.24	3.04	808	75
13	0.220	0.614	0.26	3.01	788	74
14	0.125	0.347	0.15	2.65	655	74
15	0.212	0.549	0.30	2.90	703	69
16	0.175	0.471	0.23	2.72	455	72
17	0.177	0.519	0.18	2.61	475	78
Mean	0.150	0.412	0.19	2.23	635	73

Data - Thatcher wheat, Series C (Loess -Plus Clover),
Creston, 1937.

Sample No.	Sulphur %	Cytosine %	Methionine %	Nitrogen %	Loaf Volume	Total Volume
1	0.107	0.256	0.14	2.48	705	93
2	0.121	0.240	0.13	2.42	680	89
3	0.150	0.245	0.13	2.49	705	89
4	0.179	0.302	0.23	2.44	695	96
5	0.111	0.248	0.15	2.44	705	92
6	0.113	0.251	0.09	2.44	675	89
7	0.117	0.252	0.22	2.48	675	88
8	0.130	0.257	0.24	2.48	685	88
9	0.139	0.245	0.15	2.40	670	90
10	0.110	0.271	0.13	2.48	705	92
11	0.149	0.259	0.17	2.41	685	92
12	0.206	0.260	0.21	2.48	680	92
13	0.230	0.244	0.20	2.50	700	92
14	0.125	0.247	0.09	2.39	685	92
15	0.128	0.249	0.20	2.40	705	89
16	0.175	0.274	0.20	2.46	695	92
17	0.179	0.249	0.23	2.41	695	91
Mean	0.150	0.252	0.19	2.45	694	92

TABLE XXVII

Analysis of Variance
Series C wheat, Breton, 1947.

Variance due to	D.F.	Mean squares			
		Sulphur	Cystine	Methionine	Nitrogen
Treatment	16	0.002771**	0.023468**	0.007044**	0.04365**
Error	17	0.0000182	0.0000941	0.000976	0.000347
Total	33				

** Significant beyond 1% point.

Minimum significant differences for treatment are:

0.009% sulphur.

0.04% nitrogen.

0.019% cystine.

0.06% methionine.

TABLE XXVIII

Correlation and regression coefficients,
Thatcher wheat, Series C, Breton, 1947.

r_{CS}	= 0.959**	r_{CV}	= 0.153
$r_{CS.N}$	= 0.946**	$r_{CV.N}$	= 0.046
r_{CN}	= 0.248	r_{MV}	= 0.30
$r_{CN.S}$	= −0.020	$r_{MV.N}$	= 0.23
r_{MS}	= 0.76**	r_{SV}	= 0.221
$r_{MS.N}$	= 0.75**	$r_{SV.N}$	= 0.117
r_{MN}	= 0.21	r_{NV}	= 0.458
$r_{MN.S}$	= 0.02	$r_{NV.M}$	= 0.421
r_{MC}	= 0.55*	$r_{VN.S}$	= 0.430
r_{SN}	= 0.264	$r_{VN.C}$	= 0.438

b_{CS} = 0.279%C/0.1%S.

b_{CM} = 0.12%M/0.1%S.

*Significant beyond the 5% point.
**Significant beyond the 1% point.

C = % cystine in wheat.

S = % sulphur in wheat.

N = % nitrogen in wheat.

M = % methionine in wheat.

V = Loaf volume, cc's.

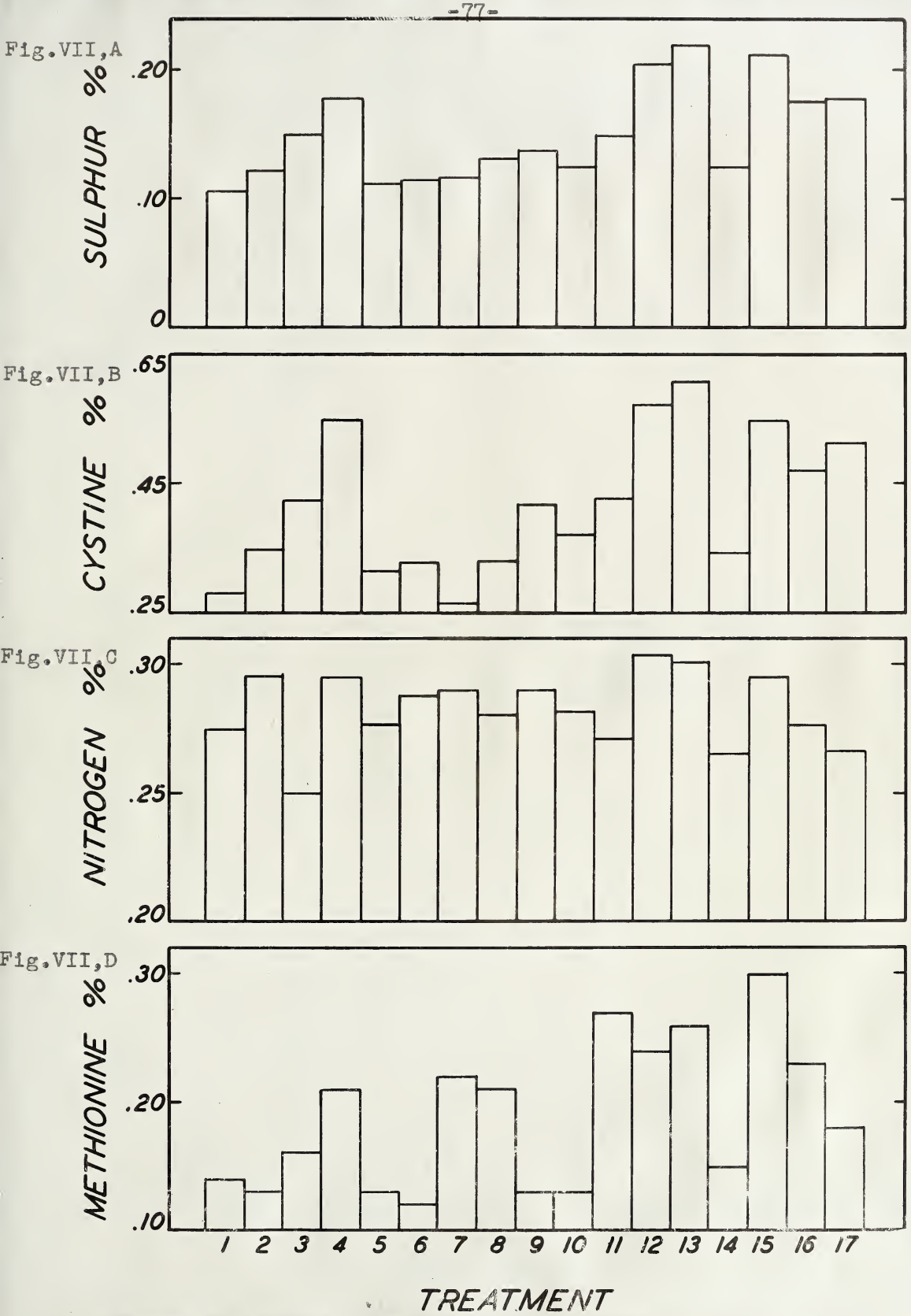


Figure VII - Effect of fertilizers on Series C wheat, 1947.

Series C wheat, Breton, 1947.

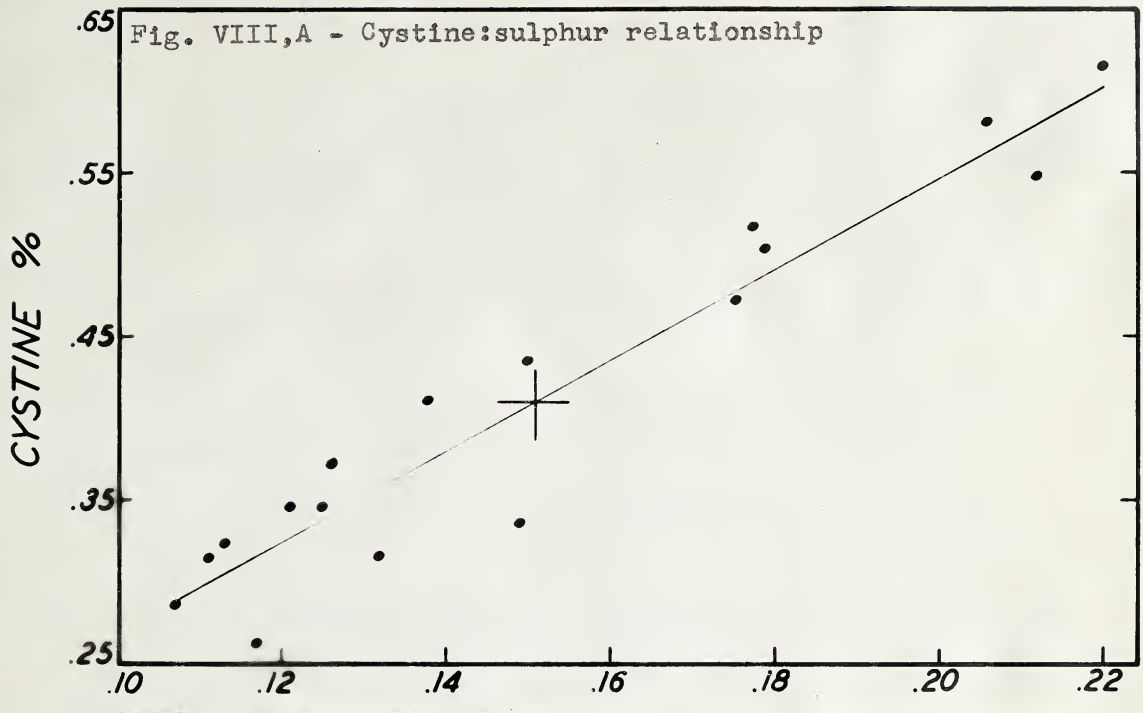


Fig. VIII,A - Cystine:sulphur relationship

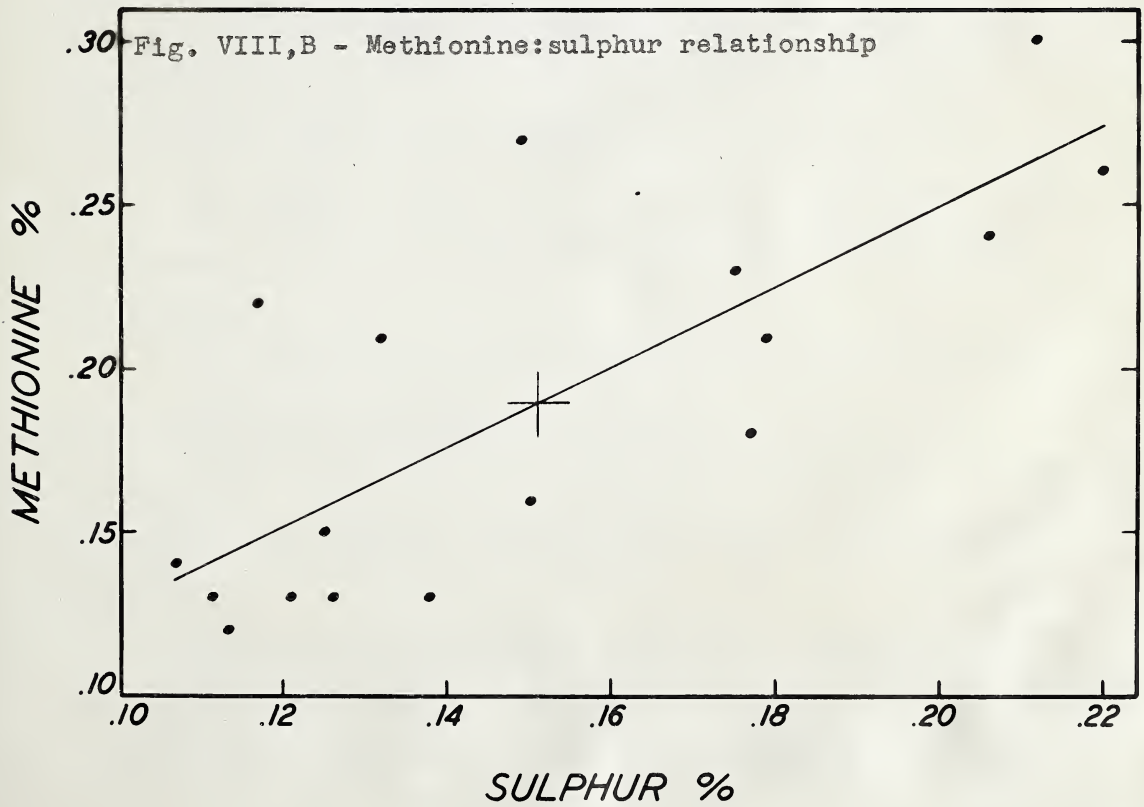


Fig. VIII,B - Methionine:sulphur relationship

Figure VIII, D and B, indicates that there is no relationship between the cystine and nitrogen contents of this wheat. The simple and partial correlation coefficients r_{CN} and $r_{CN.S}$ confirm the lack of relationship between cystine and nitrogen.

The methionine contents of the wheat after clover in the 1947 crop are distributed over a considerable range. Figure VII, A and D, and the correlation coefficients in Table XXVIII show that the methionine content of the wheat is related to the sulphur content. The scatter of methionine and sulphur values and the regression line are plotted in figure VIII, B. The correlation coefficients r_{MN} and $r_{MN.C}$ and figure VII show that the nitrogen content of wheat has no effect on the methionine content.

The simple correlation coefficient r_{MC} suggests that the amounts of methionine and cystine in the wheat proteins are related.

The correlation coefficients between loaf volume and the various wheat constituents determined are tabulated in Table XXVIII. There are no significant relationships between loaf volume and sulphur, cystine, methionine or nitrogen in wheat.

The cystine and nitrogen contents of Series C flour are given in Table XXIX. Sulphur determinations were not carried out on this series. It would be expected, however,

that the correlation between the sulphur and cystine in the flour is highly significant.

Table XXIX also gives the cystine in the flour as a percentage of that present in the wheat. The values are calculated assuming a 65% flour extraction. The range and mean of the cystine in the flour as reported are very similar to the values reported for the 1946 wheat.

The cystine content of Series C flour, as in Series E and B, is generally lower than that of the wheat. The highly significant correlation coefficient between the cystine contents of wheat and flour presented in Table XXX shows that changes in wheat cystine are accompanied by similar changes in flour cystine.

The correlation coefficients calculated for Series C flour are presented in Table XXX.

The simple correlation between cystine and nitrogen indicates a relationship between this amino acid and nitrogen. The partial correlation coefficient between cystine and nitrogen with the effects of sulphur removed is not calculated. It is likely that this statistic would be non-significant.

The loaf volumes of the samples are highly correlated with the nitrogen content of the flour. The correlation of nitrogen in the wheat and loaf volume, as previously pointed out, is not significant. This shows that there are quantities of nitrogen in the mill by-products which are not related to loaf volume. These quantities of nitrogen are sufficiently

great to offset the loaf volume-nitrogen relationship observed in the flour.

TABLE XXIX

Data - Flour from Thatcher Wheat,
Series C, Breton, 1947.

Sample No.	Cystine %	Nitrogen %	% Cystine in flour of total in wheat.
1	0.330	2.37	75
2	0.440	2.62	82
3	0.402	2.60	60
4	0.310	2.27	40
5	0.243	2.58	50
6	0.277	2.62	56
7	0.301	2.66	75
8	0.339	2.70	67
9	0.296	2.67	47
10	0.267	2.36	41
11	0.294	2.56	56
12	0.546	2.86	61
13	0.500	2.89	53
14	0.299	2.53	56
15	0.492	2.84	58
16	0.439	2.31	61
17	0.393	2.38	49
Mean	0.363	2.58	58

great to offset the Lord Talbot-Ellenborough relationship developed in the Plot.

TABLE XXIX

Data - Flour from Tankard Mill, Kaplan 5, Sweden, 1917.

Sample No.	Cyanine %	Nitrogen %	% Cyanine in flour of total in sample.
1	0.350	2.37	15
2	0.440	2.52	18
3	0.470	2.60	18
4	0.710	2.87	25
5	0.815	2.90	28
6	0.277	2.66	10
7	0.301	2.00	15
8	0.330	2.10	16
9	0.280	2.07	14
10	0.267	2.76	10
11	0.291	2.62	11
12	0.350	2.80	13
13	0.500	2.87	18
14	0.370	2.82	13
15	0.402	2.68	15
16	0.493	2.91	17
17	0.293	2.70	11
Mean	0.361	2.50	15

TABLE XXX

Correlation Coefficients
Flour from Thatcher Wheat,
Series C, Breton, 1947

r_{CN}	= 0.487*
r_{CV}	= 0.389
r_{NV}	= 0.874**
$r_{CV.N}$	= −0.073
$r_{NV.C}$	= 0.851**
r_{CW}	= 0.766**

C = % cystine in flour.
W = % cystine in wheat.
N = % nitrogen in flour.
V = loaf volume, cc's.

Rigby, as was previously stated, found a high correlation between sulphur content of wheat and cystine content of gluten. Further, he found that both these constituents were significantly correlated with loaf volume. In the material reported on in this paper there is no significant correlation between the sulphur in wheat and loaf volume. It was of interest to see if the constituents of gluten were correlated with loaf volume.

The sulphur, cystine, methionine and nitrogen were determined on the gluten washed from Series C flour. The

results are reported in Table XXXI. The cystine-sulphur, as a percentage of the total sulphur values is also included in the table.

A simple analysis of variance was carried out on each of the constituents determined. The results are reported in Table XXXII.

The calculated correlation and regression coefficients are presented in Table XXXIII. The loaf volume data used for the calculations are, of course, the same as those used for the wheat correlations.

The application of sulphur-containing fertilizers to the wheat resulted in an increase in the sulphur in the crude gluten protein. The highly significant simple correlation r_{SW} in Table XXXIII shows that there is a close relationship between the sulphur contents of wheat and gluten.

Sulphur and cystine and sulphur and methionine in gluten are highly correlated. The regression coefficients and regression lines are presented in Table XXXIII and figure IX A and B respectively. The sulphur-containing amino acids are not related to the nitrogen content of the gluten. The high values for the sulphur correlation coefficients indicate that the sulphur-containing amino acids in the gluten proteins vary with variations in the total sulphur. The nitrogen correlation coefficients show that the nitrogen content of the gluten has no effect on the quantities of cystine and methionine in the gluten.

TABLE XXXI

Data - Gluten, Series C,
Breton, 1947.

Sample No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Cystine-sulphur as % of total sulphur
1	0.654	1.498	1.18	14.60	61.1
2	0.714	1.912	0.95	14.06	71.4
3	0.696	1.798	1.01	14.01	68.9
4	0.737	1.825	1.16	14.06	66.1
5	0.540	1.342	0.85	14.60	66.3
6	0.514	1.277	0.81	14.54	66.3
7	0.500	1.087	0.98	14.40	58.0
8	0.507	1.439	0.57	14.25	75.7
9	0.443	1.451	0.26	14.49	87.4
10	0.557	1.404	0.85	14.69	67.3
11	0.587	1.414	0.98	14.28	64.3
12	0.742	1.810	1.21	13.95	65.1
13	0.808	2.022	1.25	13.80	66.8
14	0.534	1.462	0.67	14.48	73.1
15	0.857	2.004	1.50	14.24	62.4
16	0.782	1.766	1.48	14.48	60.3
17	0.729	2.022	0.88	14.40	74.0
Mean				14.31	67.9

TABLE XXXII

Analysis of Variance
Gluten, Series C, Breton, 1947.

Variance due to	D.F.	Mean Square			
		Sulphur	Cystine	Methionine	Nitrogen
Treatment	16	0.031636**	0.16025**	0.19310**	0.1311**
Error	17	0.000381	0.00184	0.01804	0.00271
Total	33				

**Significant beyond 1% point.

The minimum significant differences for treatment are:

0.039% sulphur

0.080% cystine

0.27% methionine

0.10% nitrogen.

Analysis of Variance
Table 10, Section C, Section, 1957.

Variance due to	d.f.	Sum of Squares	Mean Square	Variance Ratio	Significance
Treatments	15	0.0914	0.00609	0.1938	0.1+ns
Error	17	0.5344	0.0315	0.1804	0.005**
Total	32				

**Significant beyond 1% point.

The minimum significant differences for treatments are:

0.09% moisture
0.08% crude fat
0.07% carbohydrate
0.16% nitrogen.

TABLE XXXIII

Correlation and Regression Coefficients
Series C, Gluten.

r_{CS}	= 0.893**	r_{SN}	= −0.584*
$r_{CS.N}$	= 0.833**	r_{CM}	= 0.53*
r_{CN}	= −0.644**	r_{VC}	= 0.013
$r_{CN.S}$	= −0.333	$r_{VC.N}$	= −0.482*
r_{MS}	= 0.85**	r_{MV}	= 0.03
$r_{MS.N}$	= 0.85**	$r_{MV.N}$	= −0.02
r_{MN}	= −0.35	r_{SV}	= 0.022
$r_{MN.S}$	= 0.34	$r_{SV.N}$	= −0.411
		r_{SW}	= 0.788**

b_{CS} = 0.206%C/0.1%S.

b_{MS} = 0.21%C/0.1%S.

* Significant beyond 1%
**Significant beyond 5%.

S = % sulphur in gluten

W = % sulphur in wheat

N = % nitrogen in gluten

M = % methionine in gluten

V = loaf volume, cc's.

Gluten, Series C, 1947

Fig. IX,A

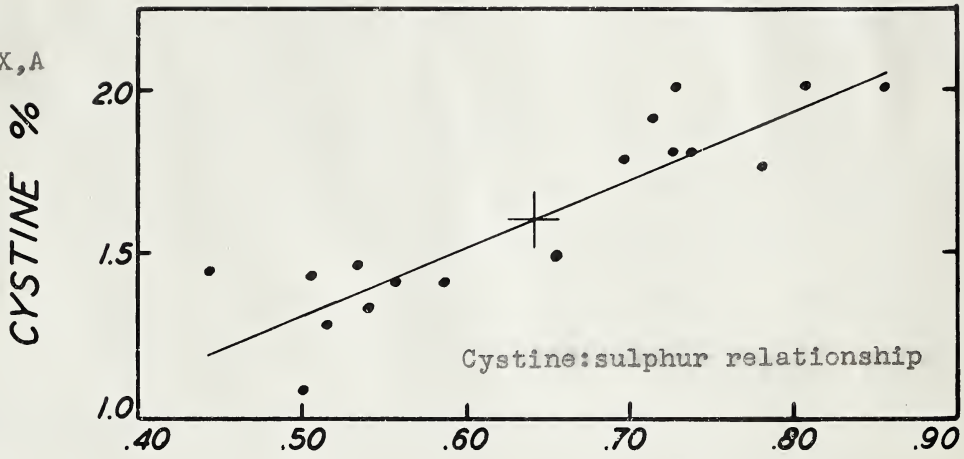
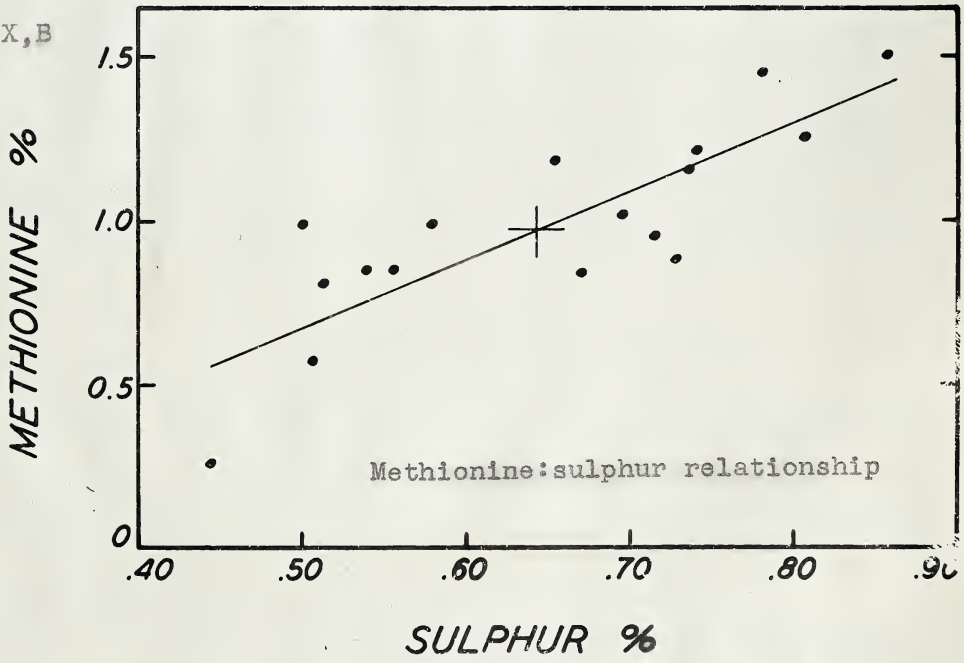


Fig. IX,B



The small range in the nitrogen content of the seventeen glutens indicates that they are similar in purity. There are small differences in the nitrogen contents of the glutens but they could be caused by differences in gluten strength. Using loaf volume as a measure of gluten strength, small volumes indicate weak glutens and large volumes strong glutens. The weak glutens readily give up starch in the washing process. The strong glutens, on the other hand, retain some starch. This results in a diluted gluten protein and less nitrogen on a percentage basis.

The relationship between cystine and methionine observed in the whole wheat proteins also exists in the gluten proteins. The sulphur-containing amino acids, cystine and methionine, are probably related in the non-gluten and mill by-product fractions of wheat.

The cystine-sulphur accounts for approximately the same percentage of the total sulphur in the gluten as in the whole wheat. This would indicate that the total sulphur in the non-gluten or mill by-product proteins has a similar distribution of cystine and methionine to that in the gluten proteins.

The correlation coefficients given in Table XXXIV show that the quantities of gluten in flour expressed as wet or dry weight are closely related to baking strength. The regression lines and scatters are shown in figure X, A and B. The actual percentages of wet and dry gluten in flour are given in Table XXXV.

Fig.X,A

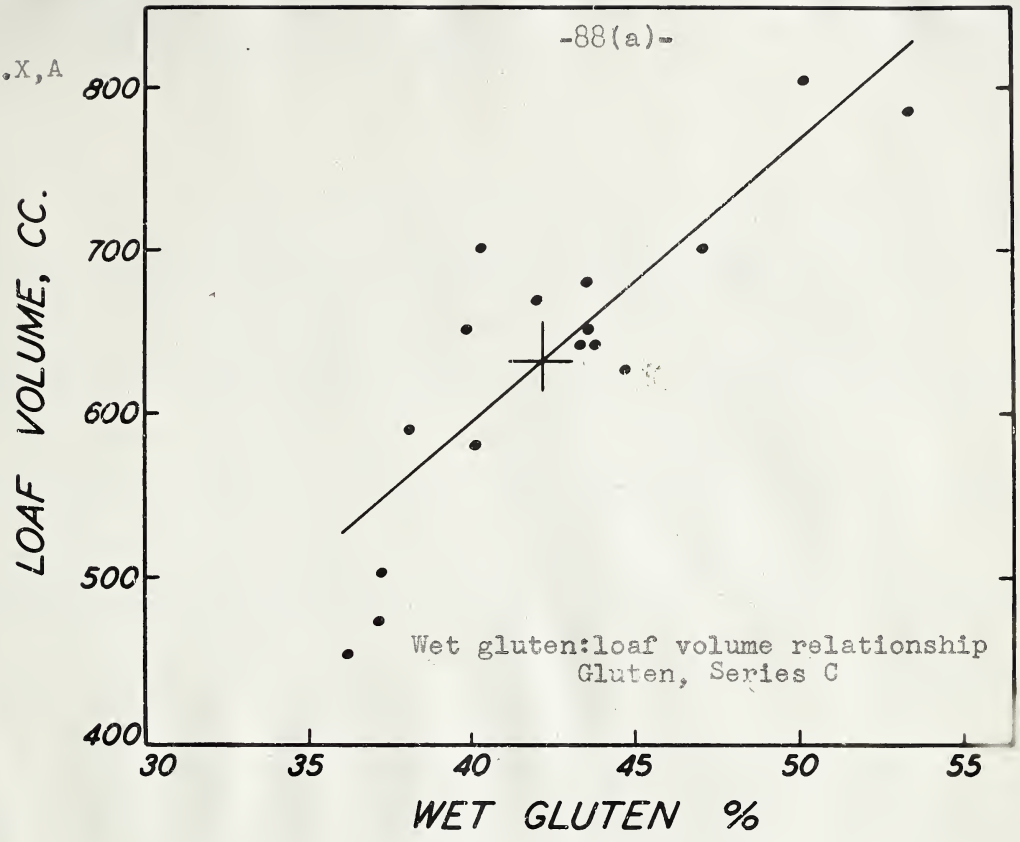
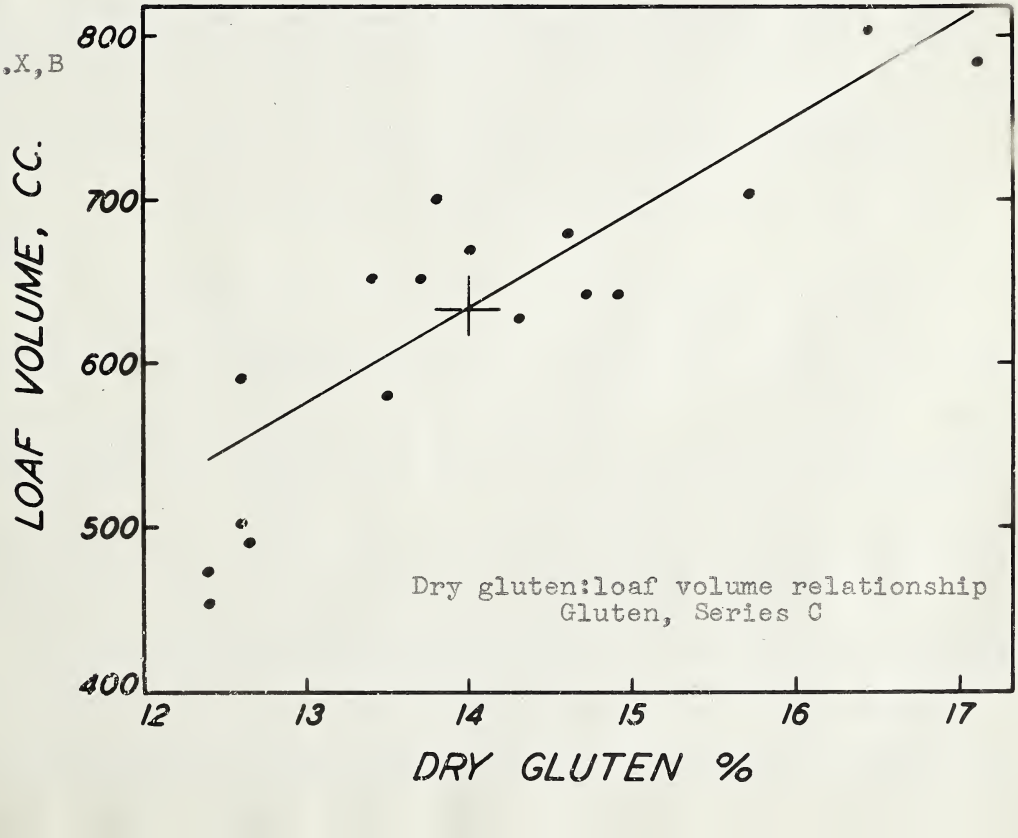

LOAF VOLUME, CC.
800
700
600
500
400
30
35
40
45
50
55
WET GLUTEN %
Wet gluten:loaf volume relationship
Gluten, Series C

Fig.X,B


LOAF VOLUME, CC.
800
700
600
500
400
12
13
14
15
16
17
DRY GLUTEN %
Dry gluten:loaf volume relationship
Gluten, Series C

The sulphur-containing amino acid content of gluten is not related to baking strength. This is shown by some of the correlation coefficients presented in Table XXXIII. The simple correlation between the quantity of cystine in the gluten of 100 grams of flour and loaf volume is significant. This value is given in Table XXXIV. The combination of the quantity of gluten and its cystine content gives a value which is significantly and positively related to loaf volume. The cystine in the gluten of 100 grams of flour is actually another measure of the quantity of gluten in the flour.

The difference between the cystine content of the flour and the cystine content of the gluten of 100 grams of flour gives a value for the non-gluten cystine in the flour. Table XXXVI gives the cystine contents of the non-gluten proteins in 17 samples of Series C flour. The correlation coefficient between non-gluten cystine and loaf volume is given in Table XXXIV.

A considerable proportion of the cystine in flour is present in the non-gluten protein. The actual values for Series C flour can be calculated from the values of Table XXXVI. The table gives the cystine in gluten as a percentage of the total cystine in the flour. The percentage cystine in the non-gluten proteins is 100 percent minus the percentage gluten cystine.

The extent of hydration of glutens of different quality will give a measure of the physical properties of the gluten. The differences in hydration of glutens of different quality as

measured by percentage moisture possibly are related to the amino acid composition of the gluten proteins. Attempts were made to relate the sulphur-containing amino acid content of gluten with the percentage moisture of the gluten.

The moisture percentages of the seventeen gluten samples from Series C flour are presented in Table XXXV. The moisture contents of these glutens were similar. The quality of the gluten as indicated by hydration is similar in the seventeen samples. There are large and significant differences in the quantity of sulphur-containing amino acids in the gluten. Therefore, in the gluten there does not appear to be a relationship between the physical properties measured by the hydration capacity and the sulphur-containing amino acid content.

TABLE XXXIV

Correlation Coefficients of gluten, Series C, 1947.

r_{WD} = 0.976**

r_{DV} = 0.869**

r_{PV} = 0.244

r_{VW} = 0.855**

r_{VF} = 0.566*

F = cystine in gluten of 100 gms. flour

P = mgms. cystine in non-gluten proteins of 100 grams flour.

V = loaf volume, cc's.

D = % dry gluten

W = % wet gluten.

* Significant beyond 5% point.
**Significant beyond 1% point.

TABLE XXXIV

Correlation Coefficients of Gluten.
Series C, 1917.

r_{WG}	= 0.9788**	Y = yield in gluten of 100 gms. flour.
r_{DG}	= 0.8694**	
r_{PG}	= 0.7417	P = mgms. protein in corresponding proteins of 100 grams flour.
r_{YG}	= 0.8978**	Y = loaf volume, c.c's.
r_{WY}	= 0.8566*	W = % dry gluten
		G = % wet gluten.

** Significant beyond 1% point.
* Significant beyond 5% point.

TABLE XXXV

Percentage of wet and dry glutens in flour and percentage moisture of gluten
Series C, 1947.

Sample No.	Wet gluten %	Weight dry gluten %	Moisture %
1	38.00	12.599	66.9
2	43.46	14.592	66.4
3	43.20	14.795	65.7
4	39.30	12.615	66.1
5	40.33	13.489	66.8
6	41.09	13.985	66.0
7	43.62	13.699	68.6
8	43.79	14.752	66.3
9	44.66	14.282	68.0
10	38.20	12.662	66.9
11	40.39	13.779	66.0
12	50.05	16.445	67.2
13	53.41	17.105	68.0
14	39.59	13.362	66.3
15	47.09	15.682	66.7
16	36.90	12.402	66.4
17	37.23	12.425	66.6

TABLE XXXVI

Gluten and non-gluten cystine
in Series C flour.

Sample No.	Cystine* in gluten	Mgms. cystine** in non-gluten proteins
1	57.3	141
2	63.4	161
3	66.2	136
4	74.2	80
5	74.5	62
6	64.6	98
7	49.5	152
8	62.5	127
9	69.9	89
10	66.7	89
11	66.3	99
12	54.6	247
13	69.2	154
14	65.2	104
15	63.8	178
16	49.9	220
17	63.9	142

* As a percentage of cystine in flour.

**Mgms. cystine in non-gluten proteins of 100 grams flour.

Sample No.	Cysteine* in flour	Wgts. cysteine in non-gluten proteins
1	57.2	144
2	61.3	101
3	0.3	170
4	1.3	90
5	0.3	0
6	0.1	0
7	0.2	72
8	0.2	127
9	0.3	90
10	0.1	36
11	0.2	90
12	0.0	0
13	0.4	144
14	0.4	108
15	0.8	126
16	0.0	0
17	0.4	126

* As a percentage of cystine in flour.

Wgts. cystine in non-gluten proteins of 100 grams flour.

Series III - Water culture experiments

Experiments I and II, as reported under "Materials," were carried out primarily to determine if and when there is a critical period for sulphur nutrient supply in the formation of the sulphur-containing amino acids in wheat.

The results of the analysis of the wheat from Experiment I are presented in Table XXXVII.

The results cannot be analysed statistically but by observation it is evident that the cystine contents of the wheat grown on the sulphur deficient nutrient solutions are as high as those of the check. The nitrogen contents of the wheat are similar.

This preliminary experiment suggests that either the wheat plant obtains all the sulphur needed for cystine synthesis in the first two weeks of the growing period or that there is a source of sulphur that has not been controlled. The cystine content of the wheat is abnormally high. It would be expected that the sulphur content of the wheat would also be high. A rough calculation shows that there is sufficient sulphur supplied in the first two weeks to account for the sulphur content of the wheat.

The results of experiment II are presented in Table XXXVII, B. The cystine-sulphur : total sulphur ratio is also included in the table.

Series III - Zero volume experiments

Experiments I and II, as reported under "Materials," were carried out primarily to determine if not even there is a critical period for solvent retention supply in the formation of the membrane-resisting tanks solid in water.

The results of the materials of the sheet from Experiment I are presented in Table XXXVII.

The results cannot be analyzed significantly but by observation it is evident that the crystals remains of the water grown on the rubber deficient nutrient solutions are as high as those of the check. The nitrogen contents of the sheet are similar.

This preliminary experiment suggests that either the sheet plant contains all the sulphur needed for optimum growth in the first two weeks of the growing period or that there is a source of sulphur that has not been controlled. The sulphur content of the sheet is exceedingly high. It would be expected that the sulphur content of the water would also be high. A rough calculation shows that there is sufficient sulphur supplied in the first two weeks to account for the sulphur content of the sheet.

The results of experiment II are presented in Table XXXVII, 5. The crystal-volume : total volume ratio is also included in the table.

The cystine content of the wheat is less in experiment II than experiment I. The trend, however, is the same in both cases.

There is very little if any difference in the percentages of nitrogen, sulphur and cystine. The wheat from tank two appears to contain slightly more sulphur and considerably more methionine than the wheat from the other tanks. The methionine value, however, seems so far out of line that there is probably an error in the cystine or sulphur determination. The low cystine-sulphur : total sulphur ratio for tank two wheat strengthens this suggestion. The cystine-sulphur : total sulphur ratios of the other tanks are approximately the same and they resemble very closely the ratios reported in earlier experiments.

Yield data were not taken for experiment II because of heavy damage by sparrows. General observations showed that there were no marked differences in yield. At harvest the plants were approximately the same height. Yields of the tanks might aid in an explanation of the observed results. It would be expected that the sulphur deficient tanks would have a lower yield. The available sulphur would then be distributed over a smaller number of kernels. This would result in a higher sulphur content for the wheat.

TABLE XXXVII

Data - Water Cultures
Effect of reducing nutrient sulphur supply
on the sulphur, cystine, methionine
and nitrogen contents of wheat

A. Experiment I

Tank No.	Cystine %	Nitrogen %
1	0.914	5.21
2	0.920	4.77
3	0.868	4.92
4	0.941	4.69

B. Experiment II

Tank No.	Sulphur %	Cystine %	Methionine %	Nitrogen %	Ratio cystine sulphur: total sulphur
1	0.189	0.581	0.16	3.10	0.82
2	0.230	0.545	0.40	3.33	0.63
3	0.188	0.544	0.20	3.15	0.77
4	0.201	0.604	0.19	3.22	0.80

Experiments III and IV were carried out to compare the effects of variations in the sulphur and nitrogen contents of the nutrient solutions on the ultimate sulphur, nitrogen, and sulphur-containing amino acid contents of wheat.

The results of the experiments are presented in Table XXXVIII, A and B.

The high nitrogen, high sulphur nutrient solution of Experiment III produced wheat highest in sulphur, cystine and methionine. The low nitrogen, low sulphur solution produced wheat with the smallest amounts of sulphur, nitrogen and sulphur-containing amino acids. The high sulphur, low nitrogen tank and the high nitrogen, low sulphur tank produced wheat that was intermediate in sulphur, cystine and methionine. The nitrogen in the wheat of the high nitrogen, low sulphur treatment is higher than the high nitrogen, high sulphur treatment.

In view of results of previous experiments the high sulphur tank, number two, was expected to produce wheat higher in sulphur cystine and methionine than tank three. This was not the case.

Yield data could possibly explain the deviations from the expected. Yields were not taken on this material because of extensive damage and loss of plants caused by sparrows. Nevertheless plate I, under "Materials", and plate II give some indication of the yield and relative size of the wheat plants at the date of harvest.

TABLE XXXVIII

Data - Effects of variations in the sulphur and nitrogen contents of nutrient solutions on the ultimate sulphur, nitrogen and sulphur-containing amino acid contents of wheat.

A. Experiment III

Tank No.	Nutrient Solutions	Sulphur %	Cystine %	Nitrogen %	Methionine %	Ratio of Cystine sulphur: total sulphur
1	Low nitrogen Low sulphur	0.174	0.517	2.66	0.18	0.79
2	Low nitrogen High sulphur	0.235	0.658	3.64	0.27	0.77
3	High nitrogen Low sulphur	0.230	0.662	4.78	0.25	0.75
4	High nitrogen High sulphur	0.312	0.819	4.63	0.43	0.70

B. Experiment IV

1	High nitrogen Low sulphur	0.254	0.681	4.16	0.34	0.72
2	High nitrogen High sulphur	0.240	0.731	3.90	0.21	0.81

No.	Task Descriptions	Optimistic Time	Most Likely Time	Pessimistic Time	Mean Time	Variance
1	Form work team	0.5	1.0	1.5	1.0	0.11
2	Bring materials to site	0.5	1.0	1.5	1.0	0.11
3	Form work	0.5	1.0	1.5	1.0	0.11
4	Pour concrete	0.5	1.0	1.5	1.0	0.11
5	Remove forms	0.5	1.0	1.5	1.0	0.11
6	Clean up	0.5	1.0	1.5	1.0	0.11

Plate II

Relative heights of wheat
from Experiment III

Tank No. 1 2 3 4

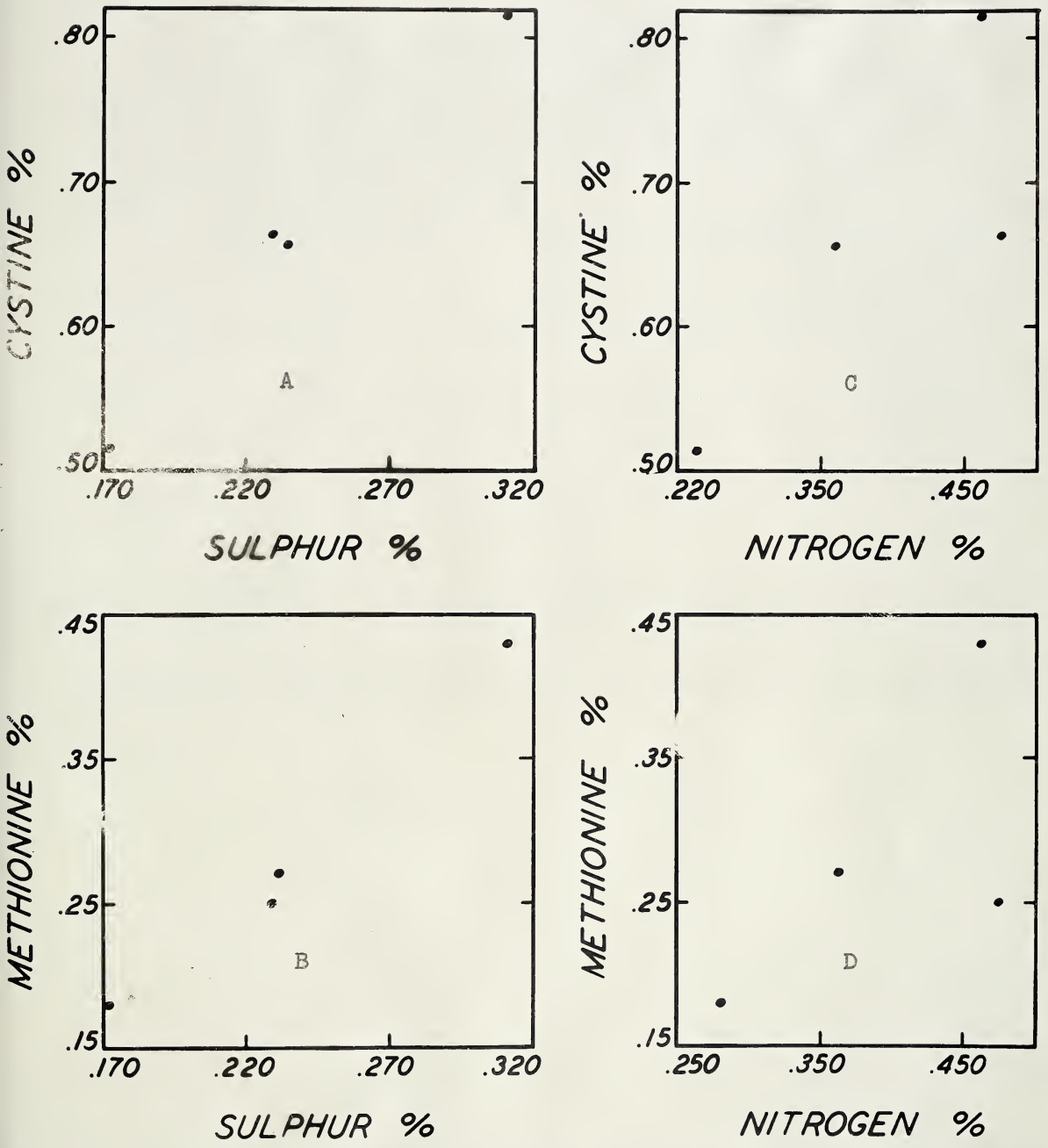
Tank No. 1 - Low sulphur, low nitrogen
2 - High sulphur, low nitrogen
3 - Low sulphur, high nitrogen
4 - High sulphur, high nitrogen

High sulphur and low nitrogen treatment produced a greater yield than the low sulphur, high nitrogen treatment. The sulphur in tank three is distributed over a smaller yield, The sulphur, sulphur-containing amino acids and nitrogen percentages are expected to be higher where the yield is reduced. This is the situation observed.

The low sulphur, low nitrogen wheat from tank one is much lower in the percentages of the determined constituents than the wheat from the other low sulphur tank (tank three). The yield of tank one is considerably greater than that of tank three and the same amounts of sulphur are distributed over a greater yield of material.

Variations in the sulphur contents of the wheat are closely related to the variations in cystine and methionine content. Figure XI, A, B, shows a close relationship between cystine and sulphur and methionine and sulphur. Figure XI, C, D, shows that the nitrogen is not closely related to the cystine or methionine contents of the wheat.

Figure XI



Relationships of cystine and methionine to sulphur and nitrogen in wheat from experiment III.

The cystine-sulphur : total sulphur ratios are generally similar to those in experiment I. With an increase in total sulphur in the wheat there is a decrease in the sulphur represented as cystine.

In experiment IV, the high nitrogen, high sulphur tank was expected to produce wheat higher in sulphur, cystine and methionine. The complete stoppage of sulphur after about two months has not resulted in any significant decreases in sulphur or methionine. The cystine value is slightly lower in the low sulphur tank. The cystine-sulphur : total sulphur ratios are similar to those in experiment III.

The results of this experiment suggest that there is a source of sulphur available to the plant which has not been recognized. Possible sources are described under "Discussion".

DISCUSSION AND SUMMARY

The development of methods for the determination of sulphur-containing amino acids in whole wheat and flour has made possible a more complete study of these constituents in wheat proteins. Rigby (34) and Gerbrandt (19) found significant correlations of the sulphur-containing amino acids in gluten with loaf volume. As this work was on gluten which contains only a part of the cystine it was desirable to carry out analysis on the non-gluten fractions of wheat.

Reserves of the material used by Gerbrandt were available for a further study of the effect of the sulphur-containing amino acids on loaf volume. Determinations of the sulphur-containing amino acids were carried out on the complete flour. The correlations with loaf volume indicate that the quantity of the crude protein of flour is a better measure of loaf volume than the percentage of cystine or methionine. The present study suggests that the method used by Gerbrandt to obtain positive correlations with the sulphur-containing amino acids is actually a correlation of gluten quantity with loaf volume.

The cystine content of Marquis flour is highly correlated with the sulphur content. There is no relationship between the total nitrogen and cystine contents of the flour. Cultural methods that will change the sulphur content of the flour will affect the cystine content to a greater degree than will changes

DISCUSSION AND SUMMARY

The development of methods for the determination of sulphur-containing amino acids in whole wheat and flour has made possible a more complete study of these constituents in wheat proteins. Loeb (1) and Dexheimer (2) found significant correlations of the sulphur-containing amino acids in gluten with loaf volume. In this work the gluten obtained only a part of the starting it was necessary to carry out analysis on the non-gluten fractions of wheat.

Because of the material used by Dexheimer were available for a further study of the effect of the sulphur-containing amino acids on loaf volume. Determinations of the sulphur-containing amino acids were carried out on the complete flour. The correlations with loaf volume indicate that the quantity of the crude protein of flour is a better measure of loaf volume than the percentage of cystine or methionine. The present study suggests that the method used by Dexheimer to obtain positive correlations with the sulphur-containing amino acids is actually a correlation of gluten quantity with loaf volume.

The cystine content of Marquis flour is highly correlated with the sulphur content. There is no relationship between the total nitrogen and cystine contents of the flour. Evidently methods that will change the sulphur content of the flour will affect the cystine content to a greater degree than will changes

in nitrogen content.

The methionine content of the Marquis flour is highly correlated with both the sulphur and nitrogen contents. The partial correlation between methionine and sulphur independent of the effects of nitrogen is non-significant. Similarly the partial correlation of methionine and nitrogen with the effects of sulphur removed is non-significant. These correlations suggest that both the nitrogen and sulphur contents of Marquis flour influence the methionine content. The sulphur and nitrogen effects on methionine are related.

The analysis of the Marquis flour suggests that the quantity of cystine and perhaps methionine could be varied with variations in the sulphur content of flour. The sulphur in flour would be expected to vary with the sulphur in wheat.

The sulphur was determined on samples of wheat grown at Breton in the continuous wheat crop series in 1946 and the wheat after clover crops for 1946 and 1947. There were significant differences in the sulphur contents of wheat as a result of fertilizer treatments. This is in agreement with the work of Peters (33). Generally the application of sulphur-containing fertilizers resulted in significantly greater increases of sulphur than the check or non-sulphur-fertilized plots.

The cystine content of the wheat followed the sulphur content very closely. The simple and partial correlation coefficients are highly significant. Changes in the nitrogen content of the wheat had no effect on the quantities of cystine present.

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The medicine content of the Durgeli flour is highly correlated with both the sulphur and nitrogen contents. The partial correlation between medicine and sulphur independent of the effects of nitrogen is non-significant. Similarly the partial correlation of medicine and nitrogen with the effects of sulphur removed is non-significant. These correlations suggest that both the nitrogen and sulphur contents of Hardis flour influence the medicine content. The sulphur and nitrogen effects on medicine are related.

The analysis of the Hardis flour suggests that the quantity of yeasting and partaking medicine could be varied with variations in the sulphur content of flour. The sulphur in flour would be expected to vary with the sulphur in wheat.

The sulphur was determined on samples of wheat grown at Dreboh in the continuous wheat crop series in 1930 and the wheat after clover crop for 1930 and 1931. There was a significant difference in the sulphur contents of wheat as a result of fertiliser treatments. This is in agreement with the work of Fabens (32). Generally the application of sulphur-containing fertilizers resulted in significantly greater increases of nitrogen than the check or non-sulphur-fertilized plots.

The cystine content of the wheat followed the sulphur content very closely. The simple and partial correlation coefficients are highly significant. Changes in the nitrogen content of the wheat had no effect on the quantities of cystine present.

In 1946 the wheat after clover crop had a much greater range in the determined constituents than the wheat from the continuous series. The legumes in the clover rotation are deep feeders. They probably bring leached sulphates closer to the surface where they are available to the wheat plant. In addition, the nitrogen content of the soil is increased because of nitrogen fixing bacteria. The greater availability of sulphur and nitrogen results in increases in the amount of cystine. The nitrogen intake of a wheat plant is of course utilized for the manufacture of numerous amino acids, proteins and other nitrogen-containing compounds. The sulphur, on the other hand, goes into the synthesis of only cystine, methionine and a few minor sulphur-containing compounds. The correlations of sulphur and cystine are therefore much more significant than the relationships between nitrogen and cystine.

The methionine content of the wheat is closely related to the sulphur uptake. The various fertilizer treatments have had little if any significant effect on the methionine contents of the wheat from the continuous series. The treatments, however, do result in large methionine differences in the wheat of the clover series in both years. Both the sulphur and nitrogen contents of the fertilized plots are higher in the wheat from the clover series. With more available nitrogen, the methionine increases with increases in sulphur. In the continuous series there is less available nitrogen, and the increases in methionine, although they are related to the increases in sulphur,

are much smaller. The regression of sulphur on methionine in the continuous series is 0.06 percent methionine/0.1 percent sulphur. The corresponding regression for the clover series is 0.13 percent methionine/0.1 percent sulphur. The available data indicate that if sufficient nitrogen is present in the soil, increases in sulphur will result in relatively large increases in methionine in wheat. If, however, the intake of nitrogen is low, increases in sulphur have little or no effect on the methionine content of the wheat.

The analyses of cystine and sulphur in wheat show that in the majority of samples the cystine-sulphur accounts for 70 to 80 percent of the total sulphur. This does not agree with the work of Gubler and Greaves (21) who estimated that approximately 50 per cent of the total sulphur in wheat is cystine-sulphur. In addition, these workers report that increases in the sulphur and cystine contents of wheat result in decreases in the sulphur represented as cystine. No such relationship was apparent in the work reported here.

The sulphur, cystine, methionine and nitrogen contents of flour are slightly lower than in the wheat. Generally there is a high correlation between the constituents in wheat and flour. The higher sulphur, cystine, methionine and nitrogen wheat samples produce flour higher in these constituents. The lower sulphur, nitrogen and amino acid wheat samples produce flour lower in these constituents.

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Of the total cystine in wheat approximately 55 to 65 percent is found in the flour as milled on an experimental mill. The variations in the methionine content of flours as a percentage of the total methionine in wheat are slightly greater than variations in the cystine. Approximately 52 to 78 percent of the methionine in wheat is present in the flour. Very few flour samples contain less than 50 percent of the cystine or methionine of the wheat. The percentage of the wheat cystine and perhaps methionine is a relatively constant value. The greatest variations in cystine are in Series B. The mean and standard deviations are 62 ± 10 per cent. The greatest variation for methionine in flour as a percentage of that in wheat is in Series E. The mean and standard deviation are 80 ± 27 per cent.

The wheat after clover crop, 1947, shows large variations in loaf volume. Rigby (34) found a high correlation between sulphur in wheat and loaf volume. His loaf volumes had variations of 347 cc. and 207 cc. for 1941 and 1942 respectively. The range in the loaf volumes of the 1947 crop is 353 cc. The analysis of Series C wheat, 1947, did not confirm the work of Rigby. Further, the sulphur-containing amino acids of the flour and wheat were not significantly correlated with loaf volume. The significant correlations of the sulphur-containing amino acids in gluten with loaf volume as reported by Rigby (34) and Gerbrandt (19) were not obtained with the 1947 material.

The cystine, methionine and sulphur contents of the

gluten washed from Series C flour were not significantly positively correlated with loaf volume. However a significant positive correlation was obtained with the cystine in the gluten of 100 grams of flour. It is obvious that this is not a specific relationship but is a measure of the total gluten in the flour. The gluten in the flour is highly correlated with loaf volume.

The correlation between methionine in gluten of 100 grams of flour and loaf volume was not calculated. Because of the highly significant correlation of the gluten content of the flour and loaf volume, and the smaller range in methionine contents of the gluten the correlation would be highly significant. Gerbrandt (19) suggested that the methionine in the gluten of 100 grams of flour was a better measure of loaf volume than the cystine content of the gluten. This would also be the situation in the 1947 flours. As the cystine contents of the glutens have a larger range than the methionine values, the combination of the cystine and gluten results gives a smaller correlation with loaf volume.

No differences were noted in the physical properties of the glutens investigated as measured by the amount of hydration. The sulphur-containing amino acids in these samples do not affect the hydration properties of the glutens.

The quantities of sulphur-containing amino acids in the gluten proteins are highly correlated with the amounts of these acids in the wheat. Fertilizer treatments that bring about changes in the cystine and methionine contents of wheat

have similar effects on the gluten. Changes in the nitrogen content of wheat have no apparent affect on the sulphur-containing amino acids in the gluten proteins.

Cystine-sulphur accounts for 67.9 ± 6.8 percent of the total sulphur in the glutens of Series C. This value is similar to the sulphur accounted for as cystine in the whole wheat and the flour. The non-gluten proteins and mill by-product proteins must therefore have approximately the same percentage of their total sulphur represented as cystine-sulphur.

The cystine in the gluten of Series C is 63.6 ± 7.0 per cent of the cystine in the flour. If 65 percent of the wheat cystine is extracted in the flour a rough calculation shows that about 40 percent of the total cystine in the wheat is distributed in the crude gluten. The other 60 percent is distributed between the non-gluten proteins of the flour and the proteins of the mill by-products.

The first two water culture experiments suggest that either the critical period for sulphur uptake is very early in the life cycle of the wheat plant or that there was a large amount of sulphur present as an impurity which was utilized by the plants.

Eaton (16) has pointed out that a plant grown on a sulphur-deficient nutrient solution could obtain sulphur from a number of sources.

Following are listed sources of sulphur, some or all of which may have had some effect on the experimental results:

(1) sulphur in the air.

(2) sulphur as an impurity in the nutrient salts.

(3) sulphur in the seed.

(4) sulphur from the fumigants used to destroy mildew and aphids in the greenhouse.

(5) sulphates present in the distilled water.

It is unlikely that a single source or even all of the sources could supply enough nutrient sulphur to raise the sulphur and sulphur-containing amino acid contents of the wheat to the high levels observed.

Eaton (16) found that soybean and sunflower seed, if grown on complete and sulphur-deficient sand cultures, did not differ in total sulphur content. The yield of the sulphur deficient cultures was much lower than the yield from the complete culture. Eaton attributes the lack of difference to the large sources of sulphur found in the air. He concludes that sulphur from the seed is not of great importance.

The cystine contents of experiment I were almost twice those of experiment II. The nutrient solutions were made up alike. The nitrogen contents of the wheat from experiment I are much higher than those of experiment II. The difference in cystine content of the wheat from the two experiments is probably a result of a quantitative effect of the protein in the wheat.

Variations in the relative sulphur and nitrogen contents of the nutrient solutions in experiments III and IV do not give

the expected differences. The yields from the different treatments apparently have some effect on the cystine, sulphur, nitrogen and methionine contents of the wheat.

Experiments III and IV show that similar relationships exist between sulphur, cystine, methionine and nitrogen at much higher levels than those observed for the Breton material.

The "differential-oxidation procedure" for the determination of cystine and methionine in wheat and flour has provided some new information. The 1947 continuous wheat series should be investigated to see if the methionine-nitrogen and methionine-sulphur relationships observed in 1946 are valid. As there are large amounts of sulphur-containing amino acids in the non-gluten flour proteins further investigations should be carried out on these proteins.

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